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Implications of ad-hoc molecular typing for infection control measures in a multi-cluster, multi-phenotypic *Serratia marcescens* outbreak in a neonatal intensive care unit

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SUMMARY

Background: *Serratia marcescens* is known to cause outbreaks in neonatal intensive care units (NICUs). Traditionally epidemiological data, antimicrobial resistance patterns and epidemiological typing have been used to guide infection prevention methods. Whole-genome sequencing (WGS) applications such as core-genome multi-locus sequence typing (cgMLST) applied during an outbreak would potentially yield more information.

Aim: To use cgMLST to acquire detailed information on the source and spread of bacteria, enabling more efficient control measures during an *S. marcescens* outbreak at a NICU.

Methods: Neonates admitted to the NICU of the Leiden University Medical Center (LUMC) during an outbreak between September 2023 and January 2024, with *S. marcescens* being cultured, were included. Environmental samples were taken to search for a common source, antibiotic susceptibility testing was performed, and antimicrobial resistance genes were analysed.

Findings: *S. marcescens* strains from 17 of the 20 positive patients were available for molecular typing. The cgMLST scheme revealed five different complex types consisting of four separate clusters. Multiple clusters made an unidentified persistent environmental source as cause of the outbreak less likely, leading to a quick downscaling of infection prevention measures. Differences were shown in aminoglycoside resistance patterns of isolates within the same complex types and patients.

Conclusion: The use of ad-hoc cgMLST provided timely data for rational decision-making during an *S. marcescens* outbreak at the NICU. Antibiotic phenotyping alone was found not to be suitable for studying clonal spread during this outbreak with *S. marcescens*.

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Introduction

Serratia marcescens is a Gram-negative rod belonging to the order Enterobacterales. From all *Serratia* species, *S. marcescens* is the species most associated with human infections. Besides asymptomatic colonization, *S. marcescens* can lead to urinary tract infections, conjunctivitis, pneumonia, but may also cause more severe infections such as sepsis or meningitis, especially in immunocompromised or critically ill patients [1]. The bacterium is known to cause outbreaks in hospital settings, especially in intensive care units (ICUs) such as neonatal intensive care units (NICUs). Especially at the NICU, outbreaks with *S. marcescens* can be challenging due to the high intensity of the care and necessary medical interventions combined with the immaturity of the immune systems of the admitted neonates.

The sources of these outbreaks with *S. marcescens* are occasionally identified. The hands of healthcare workers are often involved in the spread during an outbreak, although contamination with *S. marcescens* of environmental sources (e.g. medical devices, soaps, feeding, milk, sinks or medication) have been described in previous studies [2—4]. During such outbreaks, infection prevention measures (e.g. nursing of patients in isolation or in cohort settings) are often implemented to inhibit further spread.

Traditional tools in searching for the source and contacts during an outbreak are the collection of epidemiological data, study of antimicrobial resistance patterns (similar phenotypes are more likely to be clones compared to isolates with different phenotypes) and molecular typing of strains. Whole-genome sequencing (WGS) has become increasingly available and is now effectively implemented in many diagnostic laboratories in developed countries. WGS techniques (such as core-genome multi-locus sequence typing (cgMLST) [5,6]) are playing an increasingly important role for infection prevention and control purposes and have a high discriminatory power. cgMLST schemes consist of a fixed set of conserved genome-wide genes, and have a high discriminatory power [7]. Previous studies using molecular typing showed that generally more than one cluster can be simultaneously identified during outbreaks in the NICU [3,4,8,9]. Typing of bacterial isolates may aid in identifying the route of contamination and help to identify environmental sources. The purpose of the current study was therefore to apply a cgMLST scheme on isolates acquired during an outbreak of *S. marcescens* in the NICU, emphasizing the added value for clinical decision-making of the rapid availability of cgMLST results in addition to traditional tools only (such as epidemiological links and antibiotic phenotypes).

Methods

Study population and outbreak definition

The study population consisted of neonates who were admitted on the NICU of the Leiden University Medical Center (LUMC) in the Netherlands between September 2023 and January 2024, and had a positive culture with *S. marcescens* during their admission. The NICU is a 25-bed level-III unit consisting of 17 single-patient rooms and four twin rooms. On average, each nurse takes care of two or three neonates, depending on the intensity of care. There are monitors in each patient room and on central places on the ward. All the nurses carry handheld devices that transmit the alarm as generated by the monitors. The definition of an outbreak was made using the historical epidemiology (2019—2023) of *S. marcescens* occurrence (carriership or infection) on the NICU department, which indicated that more than two new *S. marcescens* cases per week was evocative for the presence of an outbreak.

Clinical sample collection

For an early detection of carriage of multidrug-resistant (MDR) organisms, the NICU department of the LUMC routinely (once per week) sends oropharyngeal and rectal swabs to the microbiology department from all neonates who are admitted during that time. *S. marcescens* is identified in these routine cultures. *S. marcescens* (and other organisms) that are not MDR are not reported to clinicians, but are stored in our laboratory information system (LIMS), which can be queried to identify *S. marcescens* carriership. In addition, in case of clinical suspicion of infection, samples from the blood, eye, sputum, cerebrospinal fluid (CSF) or other relevant locations are sent in for culture when indicated. From November onwards until the end of the study period, the NICU sent in additional cultures (oropharyngeal and rectal swabs) from neonates who were ready to be discharged from the department as an additional measure taken in the advent of the outbreak.

Environmental sampling

Once the outbreak with *S. marcescens* was first noticed, environmental samples were taken on the NICU department for potential identification of a common source. High-risk objects (e.g. shared objects/products such as ultrasound systems, ventilators, breastmilk pumps or baby oil) and locations (e.g. water in sink drains/wash basins in patient rooms) were identified by infection prevention nurses and swabs were taken for culture.

Strain isolation and identification

When a culture was taken for the purpose of MDR organism detection, the samples were incubated for 12—16 h at 35 °C in BD™ Tryptic Soy Broth (Xebios Diagnostics GmbH, Düsseldorf, Germany). After that, the broth was plated on selective agars (chromID™ ESBL agar, MacConkey + tobramycin/ciprofloxacin agar, and chromID® OXA-48 agar by BioMérieux, Marcy l'Etoile, France) and incubated 35 °C for 48 h. Samples from the environment were cultured in a similar way, though the broth was plated on a cystine-lactose-electrolyte-deficient (CLED) agar (BioMérieux) in addition to the previously mentioned agars.

When a culture was taken in the case of clinical suspicion of an infection, the material was directly plated on regular agars used in our clinical diagnostic laboratory (including traditional blood agar or other selective agars) depending on the origin of the specimen. The *S. marcescens* isolates were identified for clinical purposes using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry [10].

Molecular typing

To examine whether the isolates were outbreak-related, samples were analysed by cgMLST [6]. In case of the presence of different antibiotic phenotypes within one patient, all discrepant *S. marcescens* isolates from the same patient were used for typing. Using the QIAamp DNA Blood Mini Kit (Qiagen Benelux BV, Venlo, Netherlands), DNA was extracted and purified from cultured *S. marcescens* isolates. Next Generation Sequencing (NGS) libraries were prepared with the Illumina DNA Prep (Illumina, San Diego, CA, USA) and sequenced with the Illumina MiniSeq platform with 2×150 bp chemistry (Illumina). Using Ridom™ SeqSphere+ and PubMLST (https//pubmlst.org), fastQ-formatted sequences were extracted from the MiniSeq machine and processed further for data analysis. The cgMLST scheme consisted of 2692 target genes, and used a cluster threshold distance of 12 [11,12].

Infection prevention measures

At the NICU a multi-disciplinary working group infection prevention holds regular meetings to discuss current issues. The working group consists of nurses, physician assistants, an infection prevention specialist, and neonatologists. In the event of an outbreak these meetings are held more frequently and an outbreak management team is formed. During the course of this outbreak several infection prevention measures were temporarily instituted to prevent further spread. Educational meetings were organized for all healthcare workers at the ward regarding compliance with hand hygiene. All members of the working group also instructed colleagues during their care for neonates at the ward. In addition, the outbreak management team advised to nurse patients with positive cultures for *S. marcescens* in 'contact-isolation' (i.e. gloves and surgical gown required when entering the patient room, that were disposed before leaving the room), to do extra screening for *S. marcescens* at discharge, and to temporarily change the treatment in case of suspicion of bacterial sepsis and/or meningitis to meropenem. All parents of children admitted during this time were informed about the outbreak and measurements.

Antibiotic resistance

For all isolated *S. marcescens* strains, antibiotic susceptibility testing (minimal inhibitory concentration) was performed with the Vitek 2 AST N344 cards (BioMérieux, Inc., Durham, NC, USA). For amikacin susceptibility testing, a semiquantitative agar diffusion test method (30 μg; Oxoid, Basingstoke, UK) was used. For interpretation of susceptibility testing, definitions (susceptible, intermediate, or resistant) were used according to EUCAST [13]. For further determination of differences in antibiotic resistance phenotypes, antimicrobial resistance genes were searched using Ridom SeqSphere NCBI Antimicrobial Resistance Gene Finder Plus [14,15].

Results

Study population and outbreak definition

The historic epidemiology (2019—2023) of *S. marcescens* in the NICU is shown in Figure 1. The baseline incidence of *S. marcescens* carriership or infection ranged between 0 and 2 per week. Two consecutive clinical cases with *S. marcescens* infection within a short timeframe triggered a clinical microbiologist to perform a LIMS query to assess prevalence of *S. marcescens* carriership at the NICU ward. A suspected cluster in September 2023 was counted as the start of the outbreak, as the number of patients with *S. marcescens* carriership was more than two. In the period September 2023 to January 2024, 20 patients with *S. marcescens* were identified. From 17 patients isolates were available for molecular analysis, and were included in the study (Table I). During their stay on the NICU, all patients were nursed in single rooms; only siblings

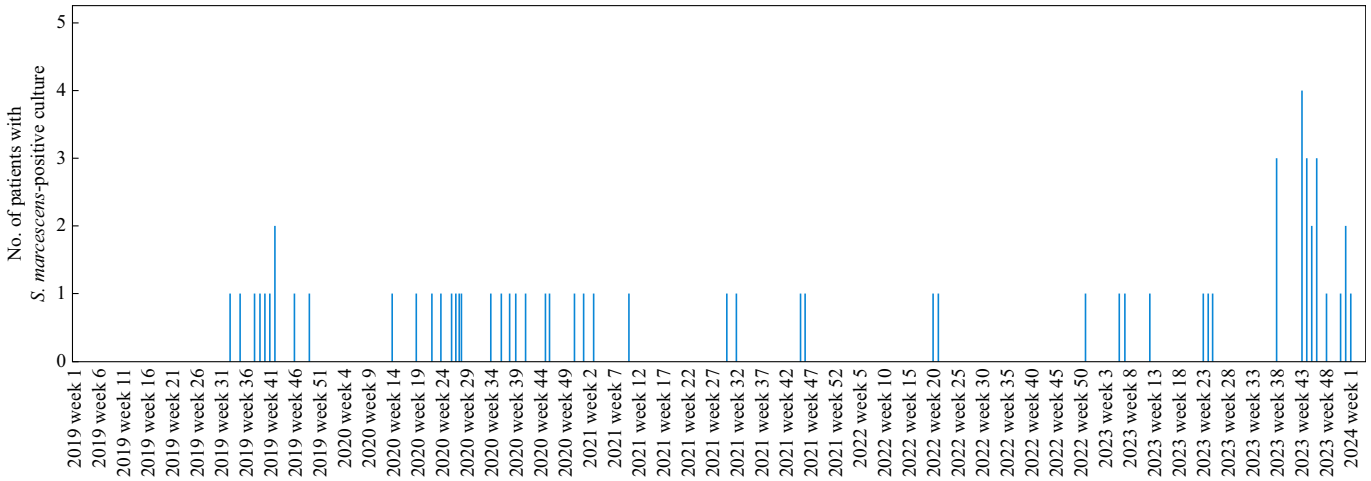

Figure 1. Weekly incidence of *Serratia marcescens* on the neonatal intensive care unit of the Leiden University Medical Center (January 2019 to January 2024).

Table I
Characteristics of the 17 neonates included in the study

Variable	Description	Mean (SD) or no. (percentage)
Gender	Male	11 (65)
Gestational age (weeks)		29 (3)
	Pregnancy duration ≤28 weeks	7 (41)
	Pregnancy duration 29–32 weeks	7 (41)
	Pregnancy duration 33–36 weeks	3 (18)
	Pregnancy duration ≥37 weeks	0
One of twins		8 (47)
Duration of NICU admission before *S. marcescens* was cultured (days)		11 (8)
Birth weight (g)		1245 (464)
Antibiotic treatment (early and late onset) before *S. marcescens* was cultured		12 (71)
Subunit NICU	Subunit 1	3 (18)
	Subunit 2	14 (82)
Born by	Vaginal labour	5 (29)
	Caesarean section	12 (71)
Radiology	Ultrasound of the brain	17 (100)
	Other (X-ray, CT, MRI)	9 (53)
Nutrition	Breast milk	17 (100)
	Intravenous nutrition	9 (82)

SD, standard deviation; NICU, neonatal intensive care unit; CT, computed tomography; MRI, magnetic resonance imaging.
Continuous variables denoted as mean (SD), categorical variables as number (percentage).

(twins) were admitted together in a double room if possible. The mean gestational age was 29 weeks. Eleven neonates (65%) were male, and eight neonates (47%) were one of twins. The mean birth weight was 1245 g, and the mean duration of NICU admission before the first culture with *S. marcescens* was identified was 10 days (range: 3–32 days). The neonates were admitted on both sides of the NICU department (18% on subunit 1, 82% on subunit 2), and the majority (71%) were born by caesarean section.

Clinical and environmental samples

The 17 included patients had 72 positive cultures (routine/clinical) for *S. marcescens* in total. None of the cultured *S. marcescens* strains were MDR (defined as either ESBL-producing, meropenem resistant, or ciprofloxacin resistant in combination with aminoglycoside resistance, or carbapenemase-producing). Most *S. marcescens* strains were isolated from the chromID ESBL agar. No additional cultures from neonates who were discharged from the department became positive for *S. marcescens*. No *S. marcescens* were cultured in the 37 cultures from environmental sampling (Supplementary Table A1).

Molecular typing

Thirty-three isolates of the 17 patients were selected for molecular typing (Table II). The minimum-spanning tree (MST) of the cgMLST scheme revealed five different Complex Types (CT) consisting of four separate clusters and one single patient with a different CT (Figure 2). The difference in target genes within clusters was ≤1. The difference in target genes between clusters ranged from 2078 to 2462. Cluster 1 (CT 1387) and cluster 3 (CT 16) both consisted of three patients. Cluster 2 (CT 1199) and cluster 4 (CT 257) were the largest clusters and both consisted of five patients. These results confirm clonal relationship and likely transmission of *S. marcescens* strains between the patients within the same cluster. The other complex type (CT 1386) belonged to a patient who was not linked to other clusters based on molecular typing, but possibly belonged to a cluster from early September of which isolates were not available for analysis. Within individual patients, all *S. marcescens* strains belonged to the same CT.

Implications of molecular typing results for clinical practice

The results of the molecular typing had significant effects on the imposed infection prevention measures. First, it became clear that neonates belonging to the same cluster were always nursed at the same subunit of the NICU (Figure 3), suggesting a decrease in compliance with hand hygiene among healthcare workers as a potential cause, allowing multiple strains of *S. marcescens* to spread simultaneously. Second, due to the fact that multiple clusters seem to coexist during the same time, it was less likely an (unidentified) environmental source was the cause of the outbreak. The combination of these findings and a decrease in the number of new cases by November/December directly led to the downscaling of infection prevention measures. The identification of four new cases (patient numbers 14–17) within two weeks by December 2023 with the same CT as patient 13 led to the re-introduction of most infection prevention measures. In the course of January, no new cases were discovered and infection prevention measures were lifted again after the last patient who had been colonized was discharged.

Table II
Characteristics of the *S. marcescens* isolates ($N = 33$) included in the study

Patient no.	Corresponding isolate number(s) in Figure 2	Outbreak investigation day	Collection site	Cluster	Complex type
1	1	1	Blood culture	No cluster	1386
2	4, 22	29	Rectal culture (2×)	Cluster 1	1387
3	8, 17	29	Eye culture, throat culture	Cluster 1	1387
4	2, 3	29	Rectal culture (2×)	Cluster 3	16
5	5, 6, 7	36	Blood culture (2×), eye culture	Cluster 3	16
6	11, 19	36	Throat culture (2×)	Cluster 2	1199
7	10, 18	42	Eye culture, rectal culture	Cluster 3	16
8	9	43	Rectal culture	Cluster 2	1199
9	12, 24	43	Rectal culture (2×)	Cluster 2	1199
10	13, 14, 20, 21	50	Rectal culture (2×), throat culture (2×)	Cluster 1	1387
11	15	50	Throat culture	Cluster 2	1199
12	16	50	Rectal culture	Cluster 2	1199
13	23	69	Sputum culture	Cluster 4	257
14	25, 26, 27	93	Rectal culture (3×)	Cluster 4	257
15	28, 29	93	Rectal culture (2×)	Cluster 4	257
16	30, 31	93	Rectal culture (2×)	Cluster 4	257
17	32, 33	100	Rectal culture (2×)	Cluster 4	257

Antibiotic resistance

Among the 33 isolates, different antibiotic phenotypes for aminoglycosides were present (Table III). In total, 12 out of 17 patients carried strains with multiple phenotypes. Differences in aminoglycoside resistance patterns (resistance and/or susceptible) also occurred within CTs (as determined with cgMLST) or within patients, suggesting that antibiotic phenotypes are not always reliable for identifying differences between strains. In all samples the aac(6′)-Ic gene was present. Single-nucleotide polymorphisms (SNPs) were present between, but not within, CTs.

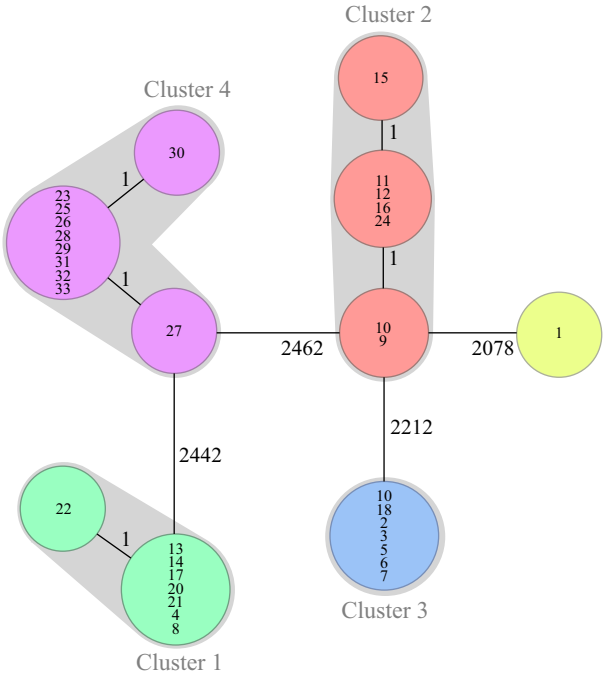

Figure 2. Minimum-spanning tree constructed using cgMLST profiles of 33 *S. marcescens* isolates that were part of an outbreak on the neonatal intensive care unit department of the Leiden University Medical Center (collected between September 2023 to January 2024). Colour, Complex Type (CT): blue, CT 16; yellow, 1386; purple, 257; green, 1387; red, 1199.

Discussion

This study describes an outbreak of *S. marcescens* involving 17 patients admitted to the NICU in a five-month period. The cgMLST scheme showed five *S. marcescens* complex types, identifying four larger clusters (three to five patients per

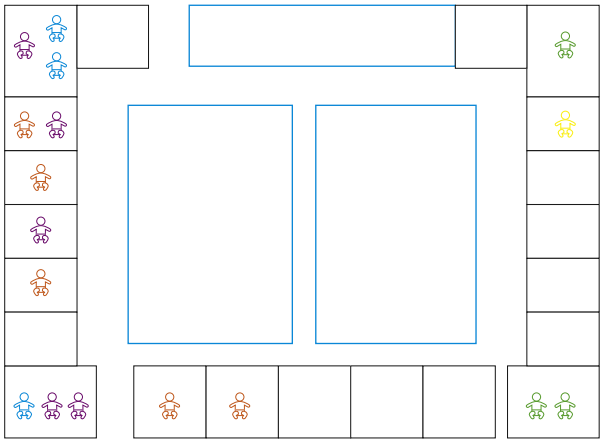

Figure 3. Schematic plan of the neonatal intensive care unit. The figures in the room represent an admission at a certain time-point during the outbreak. The colours of the figures represent the different clusters, and correspond with the cluster colours in Figure 2. The colours of the room represent either patient rooms (black) or rooms with other purposes (blue).

Table III
Overview of the isolates and their antibiotic phenotypes

No.	Complex type	Gentamicin	Amikacin	Tobramicin	Cluster
1	1386	S (MIC <1)	S (20 mm)	S (MIC <1)	–
2	16	R (MIC 6)	R (13 mm)	R (MIC >16)	4
3	16	S (MIC <1)	S (19 mm)	S (MIC <1)	4
4	1387	R (MIC 8)	R (16 mm)	R (MIC >16)	1
5	16	S (MIC <1)	–	S (MIC 2)	4
6	16	S (MIC <1)	S (19 mm)	S (MIC <1)	4
7	16	R (MIC 4)	R (14 mm)	R (MIC >16)	4
8	1387	S (MIC <1)	–	S (MIC <1)	1
9	1199	R (MIC 6)	R (13 mm)	R (MIC >16)	2
10	16	R (MIC 4)	R (13 mm)	R (MIC >16)	4
11	1199	R (MIC 8)	R (13 mm)	R (MIC >16)	2
12	1199	R (MIC 8)	R (11 mm)	R (MIC >16)	2
13	1387	S (MIC <1)	S (22 mm)	S (MIC <1)	1
14	1387	R (14 mm)	R (14 mm)	R (MIC >16)	1
15	1199	R (MIC 8)	R (13 mm)	R (MIC >16)	2
16	1199	R (MIC 8)	R (13 mm)	R (MIC >16)	2
17	1387	R (MIC 8)	R (14 mm)	R (MIC >16)	1
18	16	S (MIC <1)	–	S (MIC 2)	4
19	1199	S (MIC <1)	S (20 mm)	R (MIC 4)	2
20	1387	S (MIC <1)	S (22 mm)	S (MIC <1)	1
21	1387	S (MIC <1)	S (22 mm)	S (MIC <1)	1
22	1387	S (MIC <1)	S (23 mm)	S (MIC <1)	1
23	257	S (MIC <1)	–	S (MIC <1)	3
24	1199	S (MIC <1)	S (23 mm)	S (MIC <1)	2
25	257	S (MIC <1)	S (22 mm)	S (MIC <1)	3
26	257	R (MIC 8)	R (13 mm)	R (MIC >16)	3
27	257	S (MIC <1)	–	R (MIC 4)	3
28	257	R (MIC 8)	R (16 mm)	R (MIC >16)	3
29	257	S (MIC <1)	R (7 mm)	S (MIC <1)	3
30	257	R (MIC 8)	R (14 mm)	R (MIC >16)	3
31	257	S (MIC <1)	–	S (MIC <1)	3
32	257	R (MIC 8)	R (15 mm)	R (MIC >16)	3
33	257	S (MIC <1)	–	S (MIC 2)	3

MIC, minimum inhibitory concentration (mg/L).
The colours of the rows represent the different clusters and correspond with the cluster colours in Figure 2.

cluster). Given that the average incidence of *S. marcescens* carriership/infection was zero to two per week, a rate of more than two new *S. marcescens* cases per week was considered as the definition of an outbreak in our NICU. cgMLST showed that differences in antibiotic phenotypes (aminoglycoside resistance patterns) of *S. marcescens* were not useful for identifying routes of transmission, since different phenotypes were identified within the same complex types.

The identification of multiple clusters simultaneously was in line with several previous studies that also identified more than one clone of *S. marcescens* during the same outbreak. For example, de Boer *et al.* found 16 distinct *S. marcescens* strains among 21 cases among Dutch ICU patients within a 13-month period [8]. Also, Fleisch *et al.* identified three consecutive outbreaks caused by three genetically unrelated clones of *S. marcescens* in a NICU in Switzerland [4].

Based on the epidemiological data alone, the increase in patients with *S. marcescens* originally suggested a big outbreak from an environmental source. The existence of multiple clusters, however, suggested that a single common source was not likely the cause of the increase in incidence during the study period. As shown in Figure 1, *S. marcescens* infection or colonization from an unknown source turns out to occur occasionally in NICU patients outside an outbreak setting

(2019–2023 Q2). Contamination of the hands of healthcare workers and subsequent spread of separately introduced strains could be the cause of the coexistence of multiple clusters simultaneously. We therefore hypothesized, based on the results of the cgMLST scheme showing multiple clusters, that a decrease in the compliance with hand hygiene protocols is the most likely cause of the outbreak. What further supports this hypothesis is that neonates who were part of the same cluster were always admitted in patient rooms on the same side of the NICU. The teams taking care of the babies are being divided between the two units. The members of the teams only meet each other for breaks and in case of high workload or emergencies. Also, after extra infection prevention measures such as contact isolation for *S. marcescens* carriers and a better emphasis on compliance of hand hygiene rules were introduced, we saw a decrease in the number of new *S. marcescens* cases. Indeed, the increasing consumption of hand alcohol suggests that hand hygiene compliance increased (see Supplementary Figure A1). Of note, when the measures were lifted by December 2023, we saw an increase in new *S. marcescens* carriers again, potentially caused by reduced attention to hygiene measures. Contact isolation was immediately reinstalled, stopping any further spread in the weeks after. After lifting contact isolation once the outbreak stopped by January 2024, we did not see a new outbreak, which may be due to a general increased awareness for hand hygiene compliance.

Particularly notable were the direct clinical implications of the results of the cgMLST analysis, that is routinely available for our infection prevention department. Because the fast identification of multiple clusters made an unidentified persistent environmental source as cause of the outbreak less likely and no new unexpected cases were detected in two weeks' time, we were able to downscale the infection prevention measures and restart with regular empirical antibiotic therapy (with fewer potential side-effects and lower risks for selection of MDR organisms) in case of suspicion of infection in an early stage of the outbreak, compared to when these decisions were made with epidemiological or antibiotic phenotype data only.

Another advantage of using WGS is that we were able to analyse resistance genes –especially since previous studies have shown that differences (SNPs) in a large palindromic sequence overlapping the -35 region of the aac(6′)-Ic gene may be responsible for varying aminoglycoside susceptibilities [16,17]. In addition, plasmids encoding the aminoglycoside-modifying enzyme AAC were previously reported in aminoglycoside-resistant strains [6,17,18]. The studied intrinsic and acquired resistance mechanisms could not explain the differences we identified in aminoglycoside resistance patterns (data not shown). Other resistance mechanisms such as adaptive resistance mechanisms (e.g. overexpression of efflux pumps, biofilm production, mutation of the 16S rRNA of ribosomal proteins [17]) may be responsible for the difference in antibiotic phenotype and could be the focus of future studies looking into resistance mechanisms of *S. marcescens*.

This study has several strengths. First, detailed epidemiological information was available from the NICU which provided a better understanding of the cgMLST results.

Also, a good collaboration between different departments (neonatology, medical microbiology, infection prevention) ensured that new information about the outbreak was shared quickly, allowing rapid decision-making. An advantage of the current study population (newborns who were colonized or infected soon after their birth) is that we could exclude the possibility that some patients already had acquired *S. marcescens* prior to admission to the obstetrics department or NICU. In addition, the cgMLST scheme was available using easy-to-use and widely available software (Ridom™ SeqSphere+). The standardized approach with a high discriminatory power ensures reproducibility and the possibility of comparing results between laboratories in case of spread between different wards or hospitals.

A limitation of the study is that the source of the outbreak was never officially identified, and therefore the most likely source of contamination through the hands of healthcare workers remained an assumption. No samples were taken for culture from the hands of healthcare workers. However, the question remains whether this would have been useful since the culture would only represent the short time-period in which it was taken (a negative culture would not exclude earlier contamination with *S. marcescens*). Also, the majority of the *S. marcescens* strains were identified in surveillance cultures (not in clinical cultures) with the purpose of identifying MDR organisms in neonates. The number of *S. marcescens* cases in the outbreak could therefore have been higher compared to other hospitals that do not use such a strategy. For example, not all neonatology departments in the Netherlands have implemented routine surveillance cultures, and therefore they will only notify an outbreak when the number of invasive infections with *S. marcescens* increases. Due to the early implementation of infection prevention measures based on carriership, we have not been able to demonstrate that the increase in *S. marcescens* strains also led to more infections.

In summary, the use of cgMLST provided timely data for rational decision-making and a more efficient way of tracing the source of the *S. marcescens* outbreak in the NICU. Given the recurrent nature of *S. marcescens* outbreaks in NICUs, this is especially of added value. In addition, analysis of WGS data showed that in an outbreak with *S. marcescens*, antibiotic phenotype alone cannot be used to exclude clonal spread.

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Author contributions

M. Toorop, V. Bekker, and K.E. Veldkamp designed the research. M. Toorop, E. Wessels, S. Boers, and M. Kraakman analysed the WGS data. M. Toorop wrote the manuscript. M. Kraakman, I. Hoogendijk, J. van Prehn, H. Dogterom-Ballering, S. Boers, V. Bekker, K.E. Veldkamp, and E. Wessels revised the paper for important intellectual content.

Conflict of interest statement

None declared.

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None.

Ethical statement

The Institutional Ethics Review Board of the Leiden University Medical Center waived the need for formal approval because of the retrospective nature of the study. Infants whose parents chose to opt out of evaluation studies were excluded.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2024.05.004.

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