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Validation of a New PCR-Based Screening Method for Prevention of Serratia marcescens Outbreaks in the Neonatal Intensive Care Unit

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1 ***Research Article***

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3 ***Validation of a new PCR-based screening method for prevention of***
4 ***Serratia marcescens outbreaks in the NICU***

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14

15 **Short title**: Molecular screening for *S. marcescens*

16

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31 **Keywords:** Cohorting – Barrier nursing – Colonization screening – *Serratia*
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33

34 **Abstract**

35 **Background:** *Serratia marcescens* may cause severe nosocomial infections, mostly
36 in very low birth weight infants. Since *S. marcescens* exhibits by far the highest
37 adjusted incidence rate for horizontal transmission, it can cause complex outbreak
38 situations in neonatal intensive care units.

39 **Objective:** To establish a fast and highly sensitive colonization screening for prompt
40 cohorting and barrier nursing strategies.

41 **Methods:** A probe-based duplex PCR assay targeting the *16S rRNA* gene of
42 *S. marcescens* was developed and validated by using 36 reference strains, 14
43 *S. marcescens* outbreak- and non-outbreak isolates, defined by epidemiological
44 linkage and molecular typing, and applied in 1,347 clinical specimens from 505
45 patients.

46 **Results and Conclusions:** The novel PCR assay proved to be highly specific and had
47 an *in vitro* sensitivity of 100 gene copies per reaction (~15 bacteria). It showed a similar
48 (in laryngeal/tracheal specimens) or even higher (in rectal/stoma swabs) *in vivo*
49 sensitivity in comparison to routine microbial culture and was much quicker (<24 h vs.
50 2 d). By combining different oligonucleotide primers, there was robust detection of
51 genetic variants of *S. marcescens* strains. PCR inhibition was low (1.6%) and observed
52 with rectal swabs only. Cohort analysis illustrated applicability of the PCR assay as a
53 quick tool to prevent outbreak scenarios by allowing rapid decisions on cohorting and
54 barrier nursing. In summary, this novel molecular screening for colonization by
55 *S. marcescens* is specific, highly sensitive and substantially accelerates detection.

56 **Introduction**

57 Querying the public data base *www.outbreak-database.com* revealed that *Serratia*
58 *marcescens* is among the top pathogens of nosocomial outbreaks in neonatal intensive
59 care units (NICUs), second only to *Klebsiella spp*. Although *S. marcescens* generally
60 displays rather low virulence, it may cause severe infections, particularly in very low
61 birth weight (VLBW) infants (<1,500 g) or term neonates with inherited disorders [1]. In
62 NICUs and pediatric intensive care units (PICUs), *S. marcescens* contributes to 5-16%
63 of all nosocomial infections with positive blood culture [2, 3] (https://www.nrz-
64 hygiene.de/surveillance/kiss/neo-kiss). Data from outbreaks indicate invasive infection
65 in one out of six neonates colonized with *S. marcescens*, which is very high compared
66 to the risk in case of other pathogenic *Enterobacteriacae* [4]. Confirmed risk factors for
67 infections are VLBW, prematurity, and mechanical ventilation [5, 6]. Nosocomial
68 infection manifests most frequently as blood culture-positive sepsis (47%), pneumonia
69 (13%), and meningitis (7%), causing significant long-term morbidity and very high
70 mortality rates (30-55% of cases with positive blood culture) [7-9]. *S. marcescens*
71 exhibits an extraordinarily high adjusted incidence rate ratio (IRR 164), a measure for
72 the likelihood of a second positive blood culture with the same pathogen in a second
73 patient of the same NICU within a 30-day time period, as compared to *Klebsiella spp.*
74 (IRR 12) or *Staphylococcus aureus* (IRR 10). This rate underlines its predominance in
75 causing horizontal transmission or outbreak scenarios [10, 11].
76 After an outbreak situation causing headlines in national TV channels and newspapers
77 in 2013, the German national commission for hospital hygiene and infection prevention
78 (KRINKO) recommended a bacterial colonization screening program for neonates
79 during intensive care that included a dynamic (weekly) screening for *S. marcescens*
80 [12]. The KRINKO also emphasized the importance of developing further diagnostic
81 tools [13]. Current colonization screening is commonly based on microbial culture
82 using Columbia blood-containing agar and requires 48 h. In the NICU, however, such
83 time interval is too long for decisions on cohorting and barrier nursing.
84 Therefore, we established a specific, quick and highly sensitive PCR-based assay for
85 detection of *S. marcescens* colonization that should be tested concerning its putative
86 implication on decision-making for infection prevention and control in Neonatology.

87 **Materials and Methods**

88 For development of PCR screening method, 36 bacterial reference strains were
89 obtained representing the neonatal feces microbiome (Supplemental Tab. S1) as well
90 as 14 *S. marcescens* outbreak and non-outbreak isolates (Table 1). Furthermore,
91 1,347 rectal/stoma swabs or laryngeal/tracheal specimens from 505 patients were
92 analyzed (Fig. 1). With admission to the NICU, the patients' parents provided written
93 consent for bacterial colonization screening.

94 Genomic DNA (gDNA) was collected in eSwab regular or minitip vials (Mast
95 Diagnostica, Germany) and then released by cooking a 500 µl aliquot for 10 min at
96 98 °C and collecting the supernatant after centrifugation for 3 min at 16,100x g.

97 The sequence of the PCR-targeting region in the *S. marcescens* strains was
98 determined by Sanger sequencing (LGC Genomics, Germany) of a *16S rRNA* PCR
99 product obtained with the primers 5'-AGAGTTTGATCMTGGCTCAG-3' (forward) and
100 5'-TACGGYTACCTTGTTACGACTT-3' (reverse). Whole genome sequencing was
101 performed at the partner site of the German Center for Infection Research, University
102 of Giessen, Germany.

103 Molecular typing was performed by core-genome multilocus sequence typing
104 (cgMLST) with Ridom SeqSphere+ software version 7.7.5 (Ridom, Germany). For the
105 gene-by-gene comparison, an *ad hoc* task template scheme was established following
106 the SeqSphere+ software guide with *S. marcescens* reference genome
107 NZ_CP026050.1 (GenBank accession: GCA_000783915.2) and resulted in 3,283
108 cgMLST targets.

109 The probe-based duplex PCR assay was established, accounting for the genetic
110 diversity within the *Serratia marcescens* isolate strains. Primers and probes
111 (Supplemental Tab. S2) were synthesized (Eurofins Genomics, Germany). The PCR
112 mixture contained TaqMan Universal PCR Mastermix (#4304437, Thermo Fisher
113 Scientific), 0.4 µM *S. marcescens* primer mix, 0.4 µM *Lactobacillus spp.* primer mix,
114 0.1 µM of each TaqMan probe, 1 pg gDNA from *Lactobacillus spec.* and 1/10 Vol of
115 the processed patient sample. PCR was performed on a StepOnePlus cycler (Applied
116 Biosystems, USA) with the following protocol: 95°C for 10 min, 40 cycles of 95°C for
117 15 s, 62°C for 10 s and 72°C for 30 s. Positive and negative controls were performed
118 with each assay.

PCR specificity was evaluated with 20 ng pure gDNA of each bacterial strain (Table 1, Supplemental Tab. S1). PCR sensitivity *in vitro* was determined by serial dilutions of sequence-verified, double-stranded DNA fragments (500 bp gBlocks) covering the 16S rRNA PCR product region from *S. marcescens* and *Lactobacillus spp.*, synthesized by Integrated DNA Technologies (Coralville, USA).

Corresponding samples to the ones used for the PCR-based screening were analyzed by the standard microbial culture followed by strain identification by VITEK®2 GN ID card (bioMérieux, France) or MALDI-TOF MS (Labor Berlin, Germany).

Results

Assay design and validation of PCR analysis

The PCR assay was developed based on previously published, strain-discriminating regions of the *16S rRNA* genes of *S. marcescens* [14] and *Lactobacillus ssp.* [15], respectively, but detection of three different *S. marcescens* strain variants I-III required multiplex forward primers (Supplemental Fig. S1).

PCR specificity was evaluated using gDNA from strains representing the common microbiome in neonatal feces [16-18] and 11 non-pathogenic *Serratia* strains (Supplemental Tab. S1). Only the *16S rRNA* genes of *S. nematodiphila* and *S. ureilytica* are so closely related to *S. marcescens* genetically that their amplification could not be excluded. As those strains are neither pathogenic to humans nor to be expected in the neonatal microbiome, we accepted this cross-reactivity. Concerning the outbreak and non-outbreak isolates (Supplemental Tab. S1, Fig. 2), *16S rRNA* sequencing identified three different nucleotide variants of *S. marcescens* in the forward primer binding region: variants I-III, which were all detected by the PCR assay. To determine the genetic variability of the PCR product region in a wider variety of strains *in silico*, a sequence similarity search was performed (BLASTn) with 3,680 sequences from the taxid *S. marcescens*. The search retrieved 3,115 sequences from 2,362 different strains. In the consensus sequence, the variants I-III were confirmed in an unbiased array of isolates, suggesting a wide applicability of the PCR assay (Supplemental Fig. S1).

Since the neonatal microbiome may significantly vary upon age or antimicrobial treatment, we added 1 pg gDNA from *Lactobacillus spec.* as external inhibition control which roughly equals endogenous *Lactobacillus* amounts during the early neonatal period (≤ 7d). Hereby, Ct values ≥35 and <40 were considered “partially inhibited”, and samples were re-measured in a higher dilution. Samples with Ct values of ≥40 were considered “inhibited”.

The *in vitro* sensitivity was determined by dilution series of the PCR product to be approx. 100 gene copies per reaction (Supplemental Fig. S2). As the *S. marcescens* genome contains 7 copies of the *16S rRNA* gene, the *in vitro* detection limit of the PCR assay was approx. 15 bacteria per reaction.

Among all clinical specimens, PCR inhibitions were exclusively found in swabs of the rectum or abdominal stoma, mostly containing a high amount of feces. Inhibition occurred (101/1,347 samples = 7.5%), but mostly in *S. marcescens*-positive samples. In such cases, PCR for *S. marcescens* might have used up all reagents, thereby compromising the (*Lactobacillus spec.*-based) inhibition control PCR - leaving only 1.6% (22/1,347 samples) to be truly inhibited. This effect was also observed in the *in vitro* sensitivity analysis where the *Lactobacillus* PCR was the more often inhibited (Supplemental Fig. S2).

Application of the PCR-based screening

To determine how much quicker information on *S. marcescens* colonization was accessible by PCR diagnostics in comparison to the routine culture-based approach, 1,137 non-inhibited samples were evaluated. The median time from the sampling to the laboratory findings report was <24 h for PCR-based and 2 d for microbial culture-based diagnostics. Of note, in case of *S. marcescens* detection, the initial culture-based report usually included antimicrobial susceptibility testing results.

In the patients’ cohort, *S. marcescens* colonization by either PCR- and/or culture-based screening was detected in rectal/stoma swabs of 40 neonates (1 patient was only positive in tracheal specimens, n=41 in Fig. 1). The clinical characteristics of this cohort (Table 2) showed that colonization with *S. marcescens* mainly occurred in infants with a gestational age <32 weeks, a high frequency of mechanical ventilation and with congenital malformations, immunological disorders and/or intestinal major surgery.

181 In total, 231 samples were found to be *S. marcescens*-positive by PCR, in comparison
182 to 178 samples identified by microbial culture. This difference mainly occurred in the
183 analysis of rectal/stoma swabs with 151 positive PCR results vs. 101 positive culture
184 results (+50%). In laryngeal/tracheal specimens, the culture-to-PCR detection ratio
185 was nearly identical (80 to 77; +4%). This indicates a significantly higher sensitivity of
186 the PCR-based screening, especially for rectal/stoma swabs. As the discrepancy
187 between culture- and PCR-based analysis could result from detecting DNA of dead
188 bacteria during or after antibiotic treatment, the PCR-positive rectal specimens were
189 analyzed in depth. Among the 55 specimens (30 patients) exclusively positive by PCR
190 diagnostics, 7 out of 30 patients (23%, 11 specimens) received antibiotic treatment at
191 the time point or latest 2 days before the specimens were drawn. Antibiotic treatment
192 included 2nd line treatment with a carbapenem in 4 out of 7 of those patients. However,
193 22 out of the 40 patients (55%) PCR-positive in rectal swabs were identified in parallel
194 by culture-based screening, but 18 of those 40 patients (45%) would not have been
195 identified as colonized by *S. marcescens* at the earliest time point by only applying
196 microbiological screening. On the opposite, among all 178 specimens found to be
197 *S. marcesens*-positive in microbial culture, 5 rectal/stoma swabs (5/101 = 5 %) and
198 10 laryngeal/tracheal specimens (10/77 = 13 %) were not identified by PCR technique.
199 The contribution of pre-analytic issues remains unclear. The combined data indicate
200 that the PCR-based *S. marcescens* colonization screening in rectal/stoma swabs is
201 superior in terms of sensitivity and speed.

202 To elucidate the value of the novel screening method in managing an outbreak risk, a
203 case vignette from our level 3 NICU (Campus Charité Mitte) is illustrated in Fig. 3. This
204 ward consists of 20 (plus 4 reserve) beds in 11 rooms (3 quadruple, 4 double,
205 4 single). During an 8-month period, 83 neonates were nursed. Among them, nine
206 infants were tested positive for *S. marcescens*. In-depth analysis revealed three
207 genetically different strains of *S. marcescens;* strains A and B within a cluster, strain C
208 a single finding. From a distance, the detection dynamics did not hugely differ between
209 both screening methods. However, the detailed analysis (Fig. 4) indicated a beneficial
210 effect of the PCR-based screening. For cluster A, when the routine culture-based
211 screening was still negative in patient 5 (December 11th), the quick PCR-based
212 *S. marcescens* screening of the whole NICU revealed two other colonized patients (6
213 and 7). Patient 6 remained negative in microbial culture for additional two days, so

214 barrier precautions would not have been performed without the information from the
215 PCR-based screening. Later on, culture and PCR concordantly showed horizontal
216 transmission to a fourth patient (14, January 31st). In the other *S. marcescens* cluster
217 B, the index patient (36) was already identified by PCR-based screening at admission
218 while microbiological culture remained negative for another 14 days. At this time point,
219 horizontal transmission could already be detected in another patient (21), who was
220 negatively tested in microbiological culture for another four days. A third and fourth
221 transmission (31 and 32) were then detected simultaneously by both approaches. This
222 illustrates the potential of the quick PCR-based screening to prevent clusters or even
223 outbreak situations by timely applying cohorting and barrier nursing.
224

225 **Discussion**

226 This study describes a novel approach for the fast and reliable detection of
227 *S.marcescens*. Due to the extremely high risk of horizontal transmission, the high rates
228 of mortality, and the severe long-term morbidities in neonates infected with
229 *S. marcescens*, integrating molecular methods into the colonization screening is an
230 important progress which may extend or eventually substitute the standard screening
231 approach by microbial culture.

232 In comparison to published primer sequences [14], our qPCR assay was modified in
233 order to detect all *16S rRNA* sequence variants of *S. marcescens ssp.*, found *in vivo*
234 and *in silico*. Considering significant homologies between other *Serratia ssp*. and their
235 common presence in the human (adult) microbiome (*S. fonticola*, *S. liquefaciens, etc.*),
236 the primer set also discriminates *S. marcescens* from these *Enterobacteriaceae*.
237 Moreover, the qPCR assay exhibits high *in vitro* sensitivity with a lower limit of detection
238 of approximately 100 gene copies per reaction, corresponding to approximately 15
239 bacteria. The concept of using a duplex assay qPCR, detecting *Lactobacillus spec.* as
240 external control, allowed identification of assay inhibition, which was found in case of
241 too much fecal material in the PCR. In most cases, this issue was easily overcome by
242 subsequent analysis of a diluted specimen. One other limitation remains: Due to
243 sequence similarities, our qPCR detects non-pathogenic *S. nematodiphila* and
244 *S. ureilytica* - but this might even be an advantage as NICU outbreaks with other
245 *Serratia ssp.*, including *S. ureilytica*, have been reported [19, 20].

246 Most importantly for application in hospital hygiene, the PCR-based screening was
247 superior to microbial culture-based screening regarding both speed and sensitivity.
248 While the difference in sensitivity was marginal in the analysis of laryngeal/tracheal
249 specimens, PCR detected 50% more *S. marcescens*-positive rectal swabs. This
250 translated into a 45% higher detection rate in patients colonized with *S. marcescens*.
251 One major reason for this discrepancy could be the fact that the qPCR technique
252 cannot distinguish between DNA from living vs. non-viable bacteria. However, 5 out of
253 16 patients, whose colonization with *S. marcescens* was uniquely detected by PCR,
254 were not treated with antimicrobial substances, which argues for a truly higher
255 sensitivity of the PCR- vs. culture-based screening. In addition, a potential detection of
256 DNA from non-viable bacteria is negligible in a screening program to prevent horizontal
257 transmission in the NICU or PICU, because sustained decolonization of *S. marcescens*
258 by antibiotic treatment is rather unlikely [21, 22]. The higher speed in diagnostics may
259 be the major advantage of the PCR technique. In comparison to the microbial culture,
260 the same-day result of the PCR diagnostics is extremely valuable for cohorting and
261 barrier nursing as recommend in the German guidelines for infection prevention and
262 control in neonates [12]; even if a quicker approach using *Serratia*-selective agar plates
263 has recently become available [23]. Comparing the costs (consumables + personnel)
264 for the initial culture-based strain identification with antibiotic resistance testing vs. that
265 for PCR-based screening in the entire cohort of 505 patients enrolled in this study, the
266 novel method was about 3-fold more expensive than the routine culture. This raises
267 the question of whether the novel approach has its significance primarily in outbreak
268 scenarios or after identification of an index case as major risk of horizontal
269 transmission. Since *S. marcescens* usually do not exhibit multidrug resistance, the
270 antibiotic testing is not a prerequisite of such screening program. For a rational
271 antibiotic treatment, however, antibiotic resistance testing can be performed from the
272 asservated patient sample. Current pre-analytic use of eSwabs or minitip vials provides
273 enough material for such testing that can be initiated on the same day as obtaining the
274 PCR results, thus without delay in the gain of information. Although the case vignettes
275 indicated an advantage of the rapid colonization screening by PCR in single cases, the
276 general efficacy in preventing clusters or outbreaks with *S. marcescens* cannot be
277 estimated yet. The efficacy of the colonization screening program recommended by
278 the KRINKO is suggested by a retrospective analysis [24]. Currently, it is impossible
279 to conclude on the reduction of nosocomial sepsis or invasive infection by faster

280 decision on cohorting and barrier nursing. To overcome this limitation, a broad,
281 multicenter application of the PCR-based colonization screening is required. At its best,
282 this would be combined with PCR-based detection of other bacteria that are crucial for
283 preterm and sick term infants (such as *Klebsiella spp.* or *Acinetobacter spp.*). Such
284 PCR-based screening could be implemented as point-of-care method as currently
285 developed in adult intensive care [25]. Independent of the screening method, it is also
286 unclear yet, whether the results affect the physician's choice of the antimicrobial
287 substance in case of infection, but there is evidence that public reports on outbreak
288 scenarios lead to an increased prescription rate of third-line antibiotics in the
289 community of neonatal care [26].

290

291 **Conclusion**

292 We present a novel duplex PCR-based strategy for *S. marcescens* colonization
293 screening, which is far quicker and more sensitive than the standard microbial culture-
294 based approach. This could be an integral part in a molecular screening panel for
295 bacteria known to cause severe infections in patients admitted to the NICU or PICU.

Statements

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Statement of Ethics

With admission to the NICU, the patients' parents provided written consent for bacterial colonization screening. Additional institutional approval was not required according to German legal regulations. This has been confirmed by the Institutional Review Board of the *Charité – Universitätsmedizin Berlin*.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Author Contributions

Lina K. Sciesielski conceptualized and designed the study, collected data, carried out the analyses, and drafted and finalized the manuscript. Luisa K.M. Osang collected data, performed demographic data analyses, and contributed to the manuscript. Nicole Dinse collected data, carried out analyses, and reviewed the manuscript. Anna Weber performed bioinformatic analyses, and reviewed the manuscript. Christoph Bührer

326 contributed to the manuscript for important intellectual content. Axel Kola coordinated
327 and supervised data collection, proofed the microbial analysis and reviewed the
328 manuscript for important intellectual content. Christof Dame conceptualized and
329 designed the study, collected specimens, drafted and finalized the manuscript.

330 **Data Availability Statement**

331 The data is available from the corresponding author on request. All data generated or
332 analysed during this study are included in this article. Further enquiries can be directed
333 to the corresponding author.

References

1. Cristina ML, Sartini M, Spagnolo AM. Serratia marcescens Infections in Neonatal Intensive Care Units (NICUs). Int J Environ Res Public Health. 2019;16(4):610.
2. Gastmeier P, Loui A, Stamm-Balderjahn S, Hansen S, Zuschneid I, Sohr D, et al. Outbreaks in neonatal intensive care units - they are not like others. Am J Infect Control. 2007;35(3):172-6.
3. Mitt P, Metsvaht T, Adamson V, Telling K, Naaber P, Lutsar I, et al. Five-year prospective surveillance of nosocomial bloodstream infections in an Estonian paediatric intensive care unit. J Hosp Infect. 2014;86(2):95-9.
4. Anderson B, Nicholas S, Sprague B, Campos J, Short B, Singh N. Molecular and descriptive epidemiologyof multidrug-resistant Enterobacteriaceae in hospitalized infants. Infect Control Hosp Epidemiol. 2008;29(3):250-5.
5. Al Jarousha AM, El Qouqa IA, El Jadba AH, Al Afifi AS. An outbreak of Serratia marcescens septicaemia in neonatal intensive care unit in Gaza City, Palestine. J Hosp Infect. 2008;70(2):119-26.
6. Redondo-Bravo L, Gutiérrez-González E, San Juan-Sanz I, Fernández-Jiménez I, Ruiz-Carrascoso G, Gallego-Lombardo S, et al. Serratia marcescens outbreak in a neonatology unit of a Spanish tertiary hospital: Risk factors and control measures. Am J Infect Control. 2019;47(3):271-9.
7. Voelz A, Müller A, Gillen J, Le C, Dresbach T, Engelhart S, et al. Outbreaks of Serratia marcescens in neonatal and pediatric intensive care units: clinical aspects, risk factors and management. Int J Hyg Environ Health. 2010;213(2):79-87.
8. Ballot DE, Bandini R, Nana T, Bosman N, Thomas T, Davies VA, et al. A review of -multidrug-resistant Enterobacteriaceae in a neonatal unit in Johannesburg, South Africa. BMC Pediatr. 2019;19(1):320.
9. Piening BC, Geffers C, Gastmeier P, Schwab F. Pathogen-specific mortality in very low birth weight infants with primary bloodstream infection. PLoS One. 2017;12(6):e0180134.
10. Schwab F, Geffers C, Piening B, Haller S, Eckmanns T, Gastmeier P. How many outbreaks of nosocomial infections occur in German neonatal intensive care units annually? Infection. 2014;42(1):73-8.

11. Reichert F, Piening B, Geffers C, Gastmeier P, Bührer C, Schwab F. Pathogen-Specific Clustering of Nosocomial Blood Stream Infections in Very Preterm Infants. Pediatrics. 2016;137(4):e20152860.
12. KRINKO. Praktische Umsetzung sowie krankenhaushygienische und infektionspräventive Konsequenzen des mikrobiellen Kolonisationsscreenings bei intensivmedizinisch behandelten Früh- und Neugeborenen. 2013;Epidemiologisches Bulletin 42/2013:1-16.
13. Simon A, Dame C, Christoph J, Eckmanns T, Gärtner B, Geffers C, et al. Risikocharakterisierung intensivmedizinisch behandelter Früh- und Neugeborener und Daten zur Ist-Situation in deutschen neonatologischen Intensivpflegestationen 2013. Epidemiologisches Bulletin 42/2013. 2013;Supplement:1-56.
14. Iwaya A, Nakagawa S, Iwakura N, Taneike I, Kurihara M, Kuwano T, et al. Rapid and quantitative detection of blood Serratia marcescens by a real-time PCR assay: its clinical application and evaluation in a mouse infection model. FEMS Microbiol Lett. 2005;248(2):163-70.
15. Fu CJ, Carter JN, Li Y, Porter JH, Kerley MS. Comparison of agar plate and real-time PCR on enumeration of Lactobacillus, Clostridium perfringens and total anaerobic bacteria in dog faeces. Lett Appl Microbiol. 2006;42(5):490-4.
16. Stewart CJ, Skeath T, Nelson A, Fernstad SJ, Marrs EC, Perry JD, et al. Preterm gut microbiota and metabolome following discharge from intensive care. Sci Rep. 2015;5:17141.
17. Abrahamsson TR, Jakobsson HE, Andersson AF, Bjorksten B, Engstrand L, Jenmalm MC. Low gut microbiota diversity in early infancy precedes asthma at school age. Clin Exp Allergy. 2014;44(6):842-50.
18. Stewart CJ, Marrs EC, Magorrian S, Nelson A, Lanyon C, Perry JD, et al. The preterm gut microbiota: changes associated with necrotizing enterocolitis and infection. Acta Paediatr. 2012;101(11):1121-7.
19. Dahdouh E, Lázaro-Perona F, Ruiz-Carrascoso G, Sánchez García L, Saenz de Pipaón M, Mingorance J. Intestinal dominance by Serratia marcescens and Serratia ureilytica among neonates in the setting of an outbreak. Microorganisms. 2021;9(11):2271.
20. Karkey A, Joshi N, Chalise S, Joshi S, Shrestha S, Thi Nguyen TN, et al. Outbreaks of Serratia marcescens and Serratia rubidaea bacteremia in a central

Kathmandu hospital following the 2015 earthquakes. Trans R Soc Trop Med Hyg. 2018;112(10):467-72.

21. Strenger V, Feierl G, Resch B, Zarfel G, Grisold A, Masoud-Landgraf L, et al. Fecal carriage and intrafamilial spread of extended-spectrum β-lactamase-producing enterobacteriaceae following colonization at the neonatal ICU. Pediatr Crit Care Med. 2013;14(2):157-63.
22. Escribano E, Saralegui C, Moles L, Montes MT, Alba C, Alarcón T, et al. Influence of a Serratia marcescens outbreak on the gut microbiota establishment process in low-weight preterm neonates. PLoS One. 2019;14(5):e0216581.
23. Pérez-Viso B, Aracil-Gisbert S, Coque TM, Del Campo R, Ruiz-Garbajosa P, Cantón R. Evaluation of CHROMagar™-Serratia agar, a new chromogenic medium for the detection and isolation of Serratia marcescens. Eur J Clin Microbiol Infect Dis. 2021;40(12):2593-6.
24. Dawczynski K, Proquitte H, Roedel J, Edel B, Pfeifer Y, Hoyer H, et al. Intensified colonisation screening according to the recommendations of the German Commission for Hospital Hygiene and Infectious Diseases Prevention (KRINKO): identification and containment of a Serratia marcescens outbreak in the neonatal intensive care unit, Jena, Germany, 2013-2014. Infection. 2016;44(6):739-46.
25. Badran S, Chen M, Coia JE. Multiplex Droplet Digital Polymerase Chain Reaction Assay for Rapid Molecular Detection of Pathogens in Patients With Sepsis: Protocol for an Assay Development Study. JMIR Res Protoc. 2021;10(12):e33746.
26. Härtel C, Hartz A, Bahr L, Gille C, Gortner L, Simon A, et al. Media Stories on NICU Outbreaks Lead to an Increased Prescription Rate of Third-Line Antibiotics in the Community of Neonatal Care. Infect Control Hosp Epidemiol. 2016;37(8):924-30.

Tables

Table 1: Demographic data and clinical characteristics of neonates with *Serratia marcescens* colonization, based on rectal/stoma-positive swabs. Categorical data are given as n (%), continuous data as median (range).

Clinical Parameter	*Serratia marcescens* **positive**-tested infants (n = 40)
Female (n, %)	15 (37.5 %)
Birth weight, among them • < 500 g • 500 - 999 g • 1,000 - 1,499 g • 1,500 - 2,499 g • >2,500 g	 4 (10.0 %) 11 (27.5 %) 9 (22.5 %) 6 (15.0 %) 10 (25.0 %)
Gestational age at birth (weeks)	29.2 (24.0 – 40.6)
Term infants (n, %) Preterm infants (n, %), among them • < 28+0 weeks • > 28+0 to < 32+0 weeks *p.m.* • > 32+0 to < 37+0 weeks *p.m.*	9 (22.5 %) 31 (77.5 %) 14 (45.2 %) 12 (38.7 %) 5 (16.1 %)
Mechanical ventilation Congenital malformation Immunological disorders	25 (62.5 %) 6 (15.0 %) 5 (12.2 %)
Major surgery • intestinal • cranial • cardiac	 6 (15.0 %) 1 (2.5 %) 1 (2.5 %)
Initial *S. marcescens* screening (day of life)	3 (2-16)

Table 2: PCR-based detection of *Serratia marcescens* strain variants isolated at Charité - Universitätsmedizin Berlin between 2008 and 2013.

Strain	Origin	Year	Strain variant	PCR result
Serratia marcescens AND0213	Outbreak (colonization)	2008	**I**	**+**
Serratia marcescens AND2161	Non-outbreak (infection)	2012	**I**	**+**
Serratia marcescens AND2211	Non-outbreak (colonization)	2012	**I**	**+**
Serratia marcescens AND2261	Non-outbreak (colonization)	2012	**I**	**+**
Serratia marcescens AND2290	Non-outbreak (colonization)	2012	**I**	**+**
Serratia marcescens AND3279	Non-outbreak (colonization)	2013	**I**	**+**
Serratia marcescens AND1088	Non-outbreak (colonization)	2010	**II**	**+**
Serratia marcescens AND1234	Non-outbreak (colonization)	2010	**II**	**+**
Serratia marcescens AND2229	Non-outbreak (infection)	2012	**II**	**+**
Serratia marcescens AND3171	Non-outbreak (colonization)	2013	**II**	**+**
Serratia marcescens AND0214	Non-outbreak (colonization)	2008	**III**	**+**
Serratia marcescens AND2088	Outbreak (infection)	2012	**III**	**+**
Serratia marcescens AND2147	Non-outbreak (colonization)	2012	**III**	**+**
Serratia marcescens AND2885	Non-outbreak (colonization)	2013	**III**	**+**

Figures

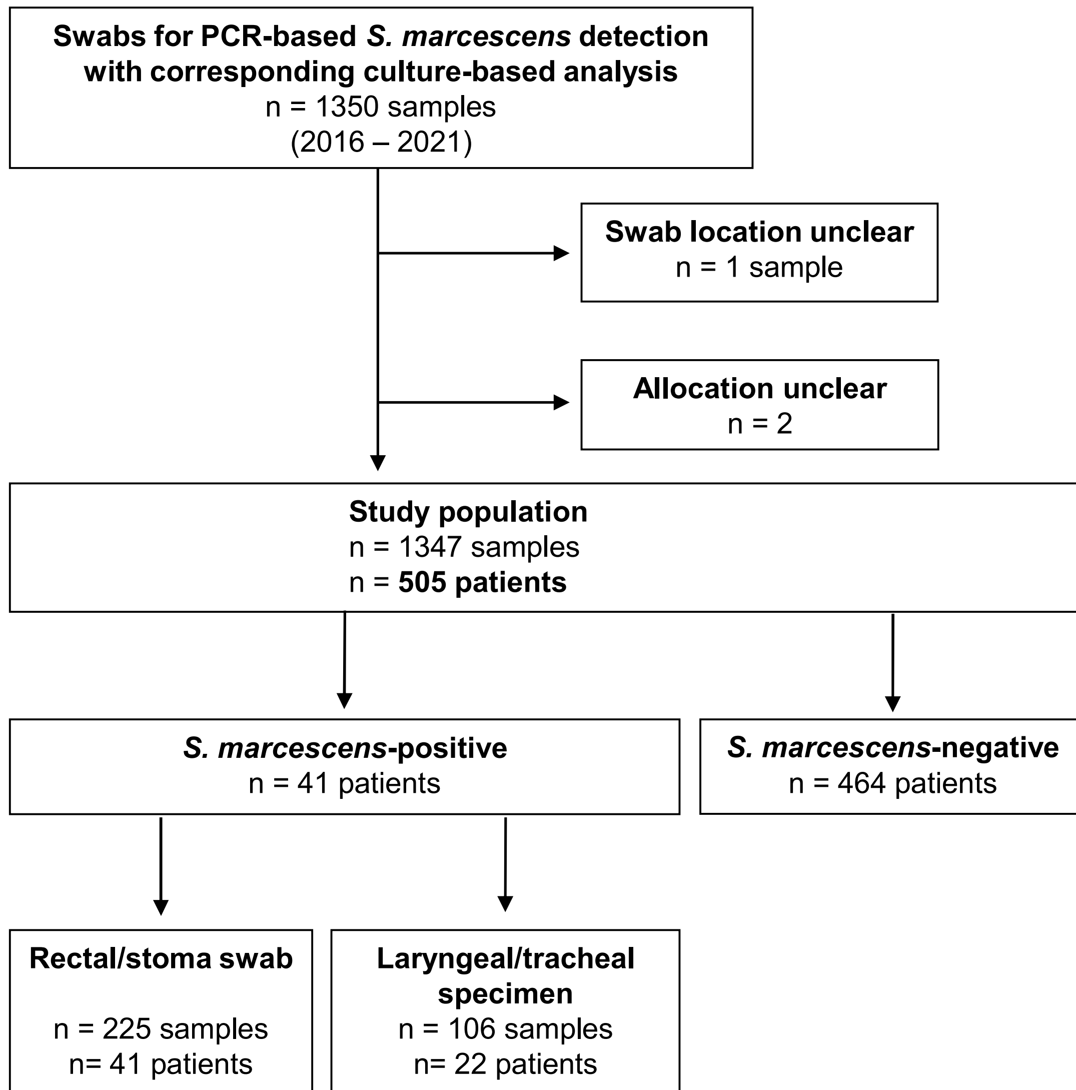


Figure 1: Study population and sample materials.

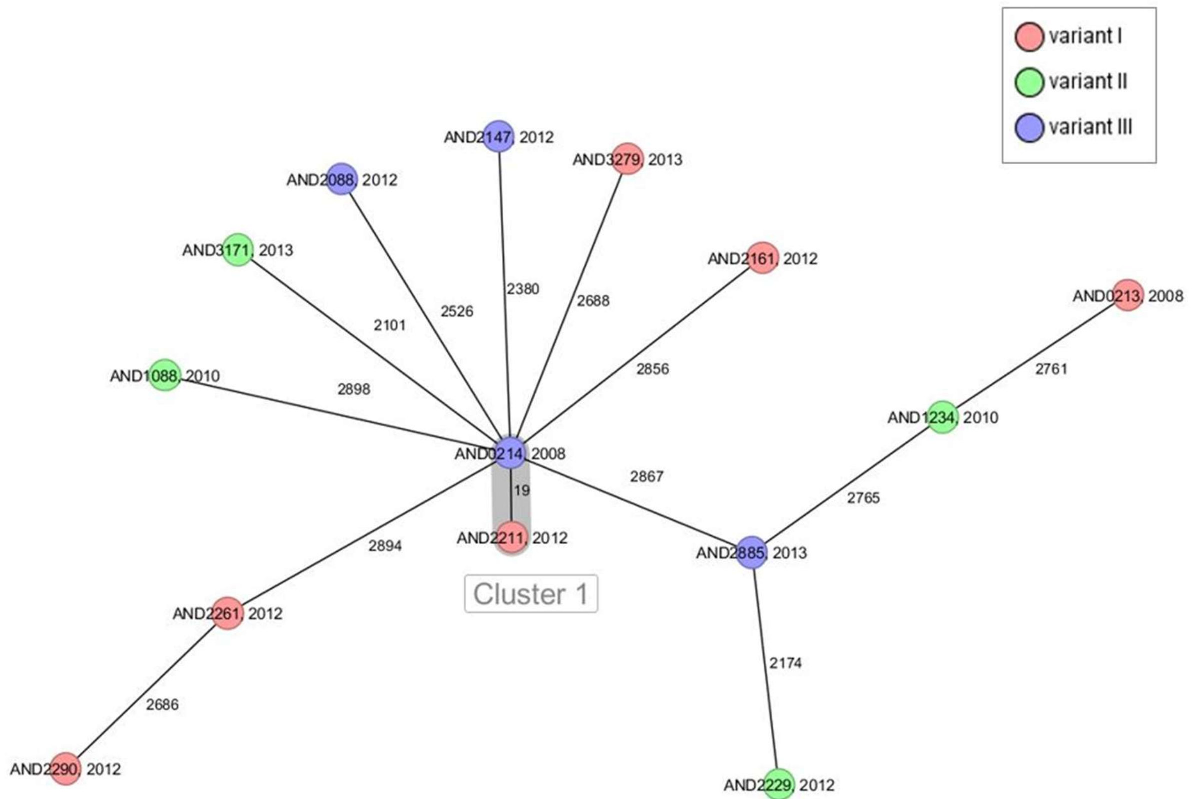


Figure 2: Molecular typing of *S. marcescens* isolates used for PCR assay validation. Minimum spanning tree based on cgMLST allelic profiles for tested strains. The circles denote the specific *S. marcescens* isolates and are colored by the nucleotide variants I-III (Supplemental Fig. S1). The connecting lines illustrate the numbers of differing alleles in the target genes. The figure shows that the tested strains were not closely related.

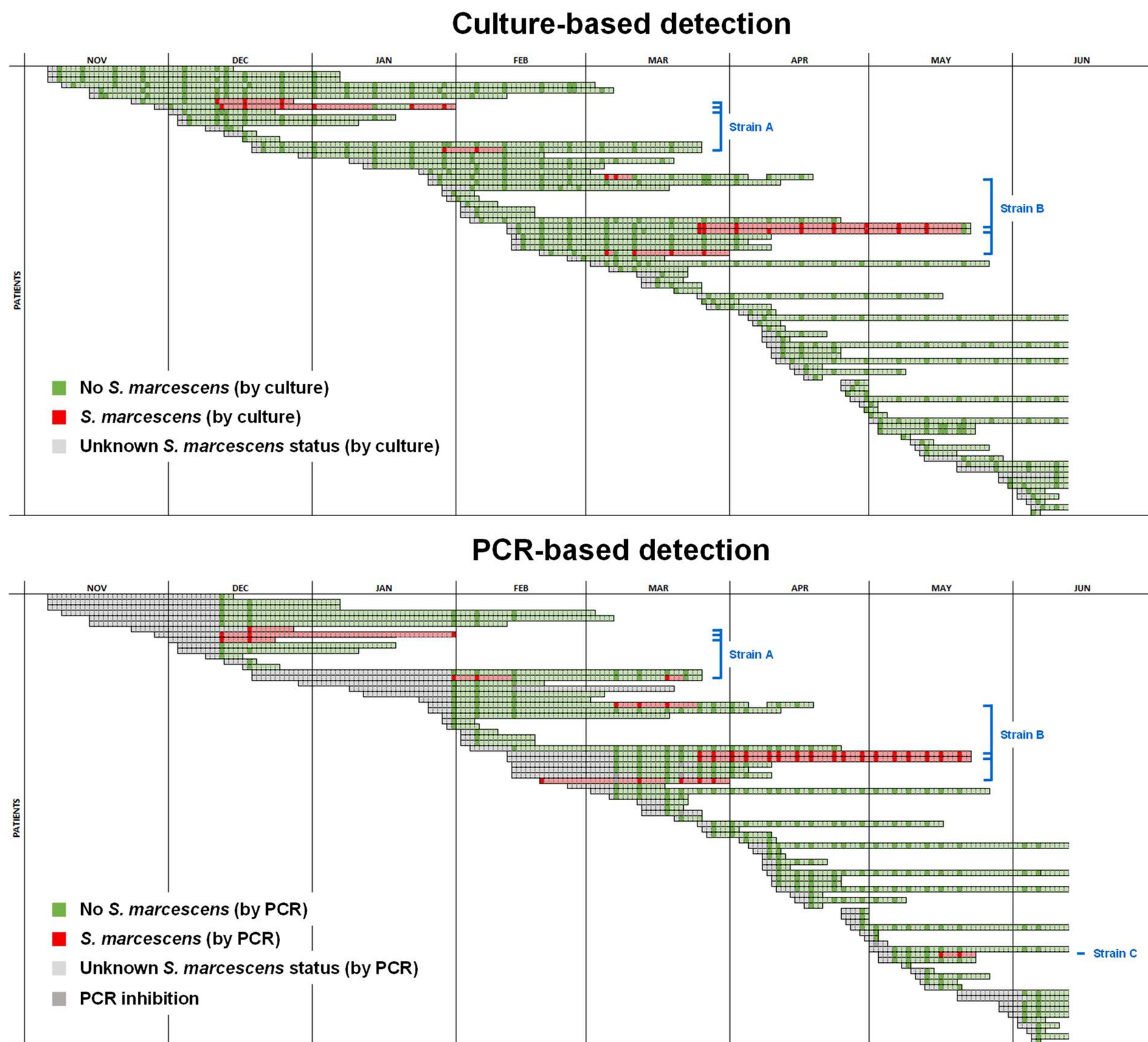


Figure 3: Case vignette of the culture- and PCR-based *S. marcescens* colonization screening in a level 3 NICU during an 8-month study period. Each box represents one patient treatment day. The color of the box indicates the screening result (all rectal swabs). The colonization status is marked as follows: unknown (gray), *S. marcescens* positive (red), *S. marcescens* negative (green). Darker color boxes

indicate the day of sampling. The colonization status coloring is retained until the next sampling result. Genetically different strains (A, B, C) of *S. marcescens* are indicated.

Figure 4: Temporal correlation of culture (C)- and PCR (P)-based *S. marcescens* screening results. Two situations are illustrated in detail: Strain clusters A (panel a) and B (panel b). Each box represents one patient treatment day. The color of the box indicates the PCR-based screening result from rectal swabs while the text indicates the culture-based Sm screening result: *S. marcescens* colonization status unknown due to PCR inhibition or lack of sample (gray), no *S. marcescens* detected (green), *S. marcescens* detected (red). Darker color boxes indicate the day of sampling. The colonization status coloring is retained until the next sampling result.

Supplementary material

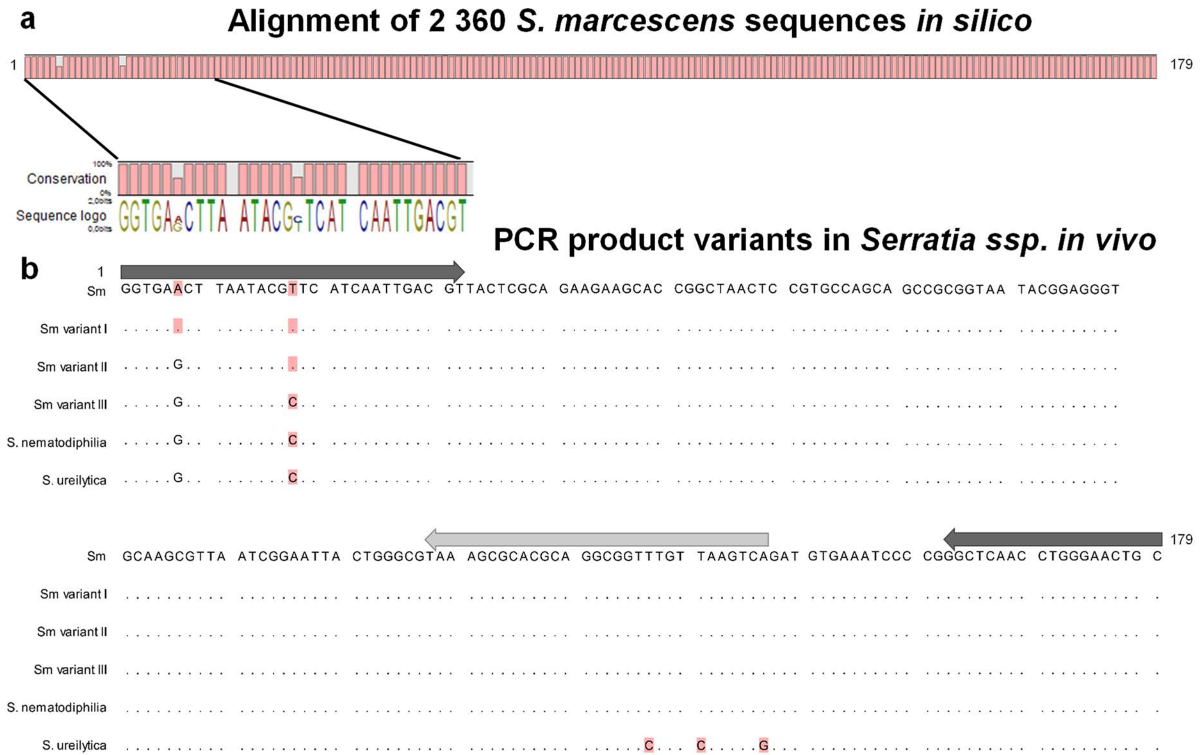
Supplemental Table S1: PCR results for reference strains from the common microbiome of the preterm and term neonate (A) and the genus *Serratia* (B).

Strain	Accession number	PCR result
A) Common preterm/newborn microbiome		
Bacteroides fragilis	DSMZ2151	-
Bifidobacterium longum subsp. infantis	DSMZ20088	-
Bifidobacterium longum subsp. longum	DSMZ20219	-
Enterobacter cloacae	ATCC13047	-
Enterobacter ludwigii	DSMZ16688	-
Enterococcus faecalis	ATCC29212	-
Escherichia coli	ATCC25922	-
Klebsiella oxytoca	ATCC700324	-
Klebsiella pneumoniae	ATCC700603	-
Lactobacillus fermentum	CECT5716	-
Lactobacillus reuteri	DSMZ17938	-
Propionibacterium acnes	DSMZ1897	-
Proteus mirabilis	IZW 538-5/2011	-
Proteus vulgaris	IZW 495-1/2011	-
Salmonella enteritidis	IZW 161-2/2015	-
Salmonella typhimurium	IZW 280-2/2016	-
Sphingomonas aromaticivorans	DSMZ12444	-
Staphylococcus epidermidis	ATCC700577	-
Staphylococcus haemolyticus	Clinical isolate	-
Stenotrophomonas maltophilia	Clinical isolate	-
Streptococcus mutans	DSMZ20523	-
Streptococcus salivarius	DSMZ20560	-
B) Genus *Serratia*		
Serratia entomophila	DSMZ12358	-
Serratia ficaria	CECT5716	-
Serratia fonticola	Clinical isolate	-
Serratia grimesii	DSMZ30063	-
Serratia liquefaciens	DSMZ4487	-

Serratia marcescens subsp. marcescens	ATCC8100	**+**
Serratia nematodiphila	DSMZ21420	**+**
Serratia odorifera	Clinical isolate	**-**
Serratia plymuthica	Clinical isolate	**-**
Serratia proteamaculans	DSMZ4543	**-**
Serratia quinivorans	DSMZ4597	**-**
Serratia rubidaea	DSMZ4480	**-**
Serratia symbiotica	DSMZ23270	**-**
Serratia ureilytica	DSMZ16952	**+**

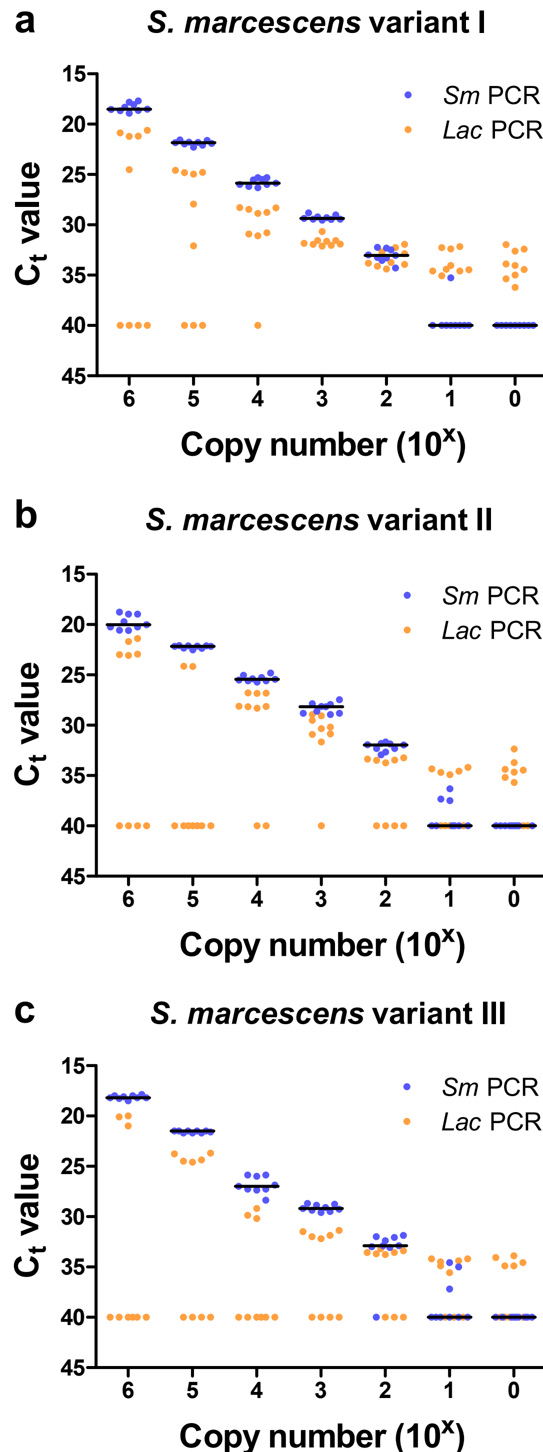
Supplemental Table S2: Primers and probes used for PCR detection of the *16SrRNA* from Serratia marcescens and *Lactobacillus spec.* Primers for detection of *S. marcescens* gDNA were adapted from Iwaya et al. [14], and for detection of *Lactobacillus spec.* from Fu et al. [15].

	Forward primer(s) (5‘→3‘)	**Probe (5‘→3‘)**	**Reverse primer (5‘→3‘)**
S. marcescens variant I	GGTG**AA**CTTAATACG**TT**CA TCAATTGACGT	TGACTTAACAAACCGC CTGCGTGCGCTTTA	GCAGTTCCCAGGTTGAGCC
S. marcescens variant II	GGTG**AG**CTTAATACG**TT**CA TCAATTGACGT		
S. marcescens variant III	GGTG**AG**CTTAATACG**CT**CA TCAATTGACGT		
Lactobacillus spp.	CGATGAGTGCTAGGTGTTG GAGG	ATTAAACCACATGCTC CACCGCTTGTGCG	CAAGATGTCAAGACCTGGTA AGGTTCTTC



Supplemental Figure S1: Specificity for detection of different genetic variants of *S. marcescens* outbreak strain types and other *Serratia spp*. (a) Consensus sequence of the PCR product from 2,362 *S. marcescens* strain sequences *in silico* and a close-up of the forward primer binding region confirming the genetic variants **I-III** in an unbiased array of isolates. **(b)** Binding sites for primers (gray) and probe (light gray)

in the *16S rRNA* sequence are indicated. For the probe and reverse primer binding sites, no genomic differences were found between the *S. marcescens* variants.



Supplemental Figure S2: Determination of *in vitro* sensitivity of the PCR assay. Ct values of the duplex PCR assay components are shown with serial dilutions of a

standardized PCR template representing the three different *S. marcescens* strain variants I **(a)**, II **(b)** and III **(c)** in blue and the *Lactobacillus* (Lac)-based inhibition control PCR in orange.