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Genome-oriented outbreak management reveals the first detection of concurrent transmissions of *Serratia sarumanii* and *Serratia bockelmannii* in a neonatal department

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SUMMARY

Aim: Non-pigmented *Serratia marcescens* — bacteria otherwise known for occasional reddish pigmentation and frequent outbreaks in neonatal intensive care units (NICUs) — were recently reclassified to novel species: *S. sarumanii* and *S. bockelmannii*. This raised questions about possible differences regarding clinical significance. Here, we report the first detected concurrent transmission of these novel species in a neonatal department.
Methods: Between March and October 2023, non-pigmented *S. marcescens* (according to mass spectrometry) were repeatedly found in 40 patients of a neonatology department. Proactive short-read ($N = 42$, including two environmental) and for three large clusters additional long-read (nanopore sequencing, $N = 23$) whole-genome sequencing (WGS) was performed, followed by ad-hoc core-genome multi-locus sequence typing (cgMLST, SeqSphere+ software), taxonomic analysis with Type Strain Genome Server (TYGS), and virulence factor/resistance prediction with Abricate.
Results: WGS revealed a polyclonal *Serratia* spp. population comprising 14 genotypes, including three large clusters ($N = 6$, 8, and 9, respectively) alongside two pairs of twins and a small cluster of three isolates. In contrast to initial MALDI-ToF classification as *S. marcescens*, WGS-driven taxonomic analysis reclassified isolates within the largest

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cluster as *S. bockelmannii* (including one environmental isolate), whereas the remaining two large clusters were assigned to *S. sarumanii*.

Conclusion: WGS-based analysis revealed a prolonged outbreak involving newly classified non-pigmented *Serratia* spp. — *S. bockelmannii* and *S. sarumanii* — through conventional mass spectrometry. This highlights the importance of routinely implementing WGS to accurately track transmission and implement effective infection control measures.

Introduction

Among *Serratia* spp., *S. marcescens* is considered of highest clinical relevance, affecting predominantly vulnerable patients such as the elderly, newborns, and immunocompromised patients. Infections caused by these Enterobacterales include bloodstream infection (BSI), conjunctivitis, and infections of the urinary or respiratory tract and have a high mortality rate (6.4%), regardless of antimicrobial susceptibility [1,2].

Historically, the pathogen gained awareness due to association with eucharistic miracles, linked to some strains' ability to synthesize prodigiosin, a characteristic red pigment with biocidal activity [3,4]. However, species attribution was recently revisited. Following whole-genome-sequencing (WGS) -based analyses, several non-pigmented strains were re-classified as different species including *S. nevei* (described in 2020), *S. montpellierensis* (described in 2024 from non-human samples), *S. bockelmannii* (described in 2020), and *S. sarumanii* (described in 2024) [5—7].

From an infection prevention and control (IPC) perspective, *S. marcescens* is highly transmissible and outbreak-prone — particularly in neonatal intensive care units (NICUs) [8—10]. Data preceding WGS-based analyses concluded that outbreaks in NICUs typically affect 23.9 newborns on average, and when identified (51.4%), outbreak sources included cosmetic products and other environmental sources (i.e., soap dispenser, baby scale, faucet aerators, water drains) [1,8,9,11,12]. However, the pathogen's negative reputation — shaped by low-resolution methods such as biochemical- or mass spectrometry-(MS) based species identification and phenotypic antimicrobial testing — may have been overestimated [13]. Rather than prolonged outbreaks, genome-oriented analyses repeatedly demonstrated multiple, smaller outbreaks within a polyclonal population, at times uncovering strain-specific environmental sources [9,10,13—15]. Despite successes that highlight the value of WGS in IPC and the need to challenge our understanding of *Serratia* spp., its availability for IPC purposes is limited.

This article (i) describes how the WGS-based investigation of a suspected large *S. marcescens* (MS-identified) outbreak refuted the assumption of a single-species outbreak, (ii) reveals the first documented concurrent outbreaks of two newly described *Serratia* spp. — *S. bockelmannii* and *S. sarumanii* — in a clinical setting, and (iii) reflects on how the implementation of genome-oriented IPC, and the hereby-mediated possibility to correctly identify novel subspecies influenced outbreak management.

Methods

Study setting and design

We performed proactive WGS-aided outbreak detection and management of a *Serratia* spp. outbreak that involved newborn patients in an NICU (14 beds), pediatric ICU (PICU, 20 beds) and adjacent neonatal stepdown unit (NStU, 30 beds). The departments specialize in treatment of preterm-born patients and patients with low and very low birth weight (VLBW).

Standard IPC at the NICU involves a 1:2 healthcare worker to patient ratio and patient-specific use of products for personal care. As recommended by the German Commission for Hospital Hygiene and Infection Prevention (KRINKO), all PICU and NICU patients and VLBW NStU patients (weight: <1500 g) are screened weekly (throat and rectal swab) for mult-idrug-resistant organisms (in Gram-negative bacteria defined as resistance to minimum piperacillin and third-generation cephalosporins) and, regardless of susceptibility pattern, *S. marcescens*, *Pseudomonas aeruginosa*, *Enterobacter* spp., and other pathogenic bacteria [16].

Outbreak management

Nosocomial *S. marcescens* (MS-identified) detection first occurred in November 2022, the index patient remaining hospitalized for over 6 months (Figure 1). In March 2023, an additional 11 cases were diagnosed over a period of 3 weeks (Figure 2). This prompted outbreak management including WGS of all isolates at University Hospital Regensburg (the initial 11 isolates, proactive thereafter), implementation of contact precautions, observation of IPC compliance, education of parents and staff on IPC, and environmental investigations (sink drains: $N = 53$, sampled twice; soap dispensers: $N = 20$; cleaning trolleys and different surfaces in the milk kitchen: $N = 22$). Thereafter, only three nosocomial cases were diagnosed between weeks 4 and 13.

In weeks 14—32, the incidence rose again ($N = 28$). The peak in weeks 14—16 ($N = 12$) triggered a doubling in routine screening frequency to twice weekly and a temporary admission stop. Initially, the screening included all multi-drug-resistant Gram-negative rods, and was then focused on *Serratia* spp. (Figure 2). Two sinks that yielded growth of *S. marcescens* (MS-identified) in week 14 were immediately replaced, and the isolates were sequenced. Intensified containment measures quickly reduced nosocomial cases to 0—2

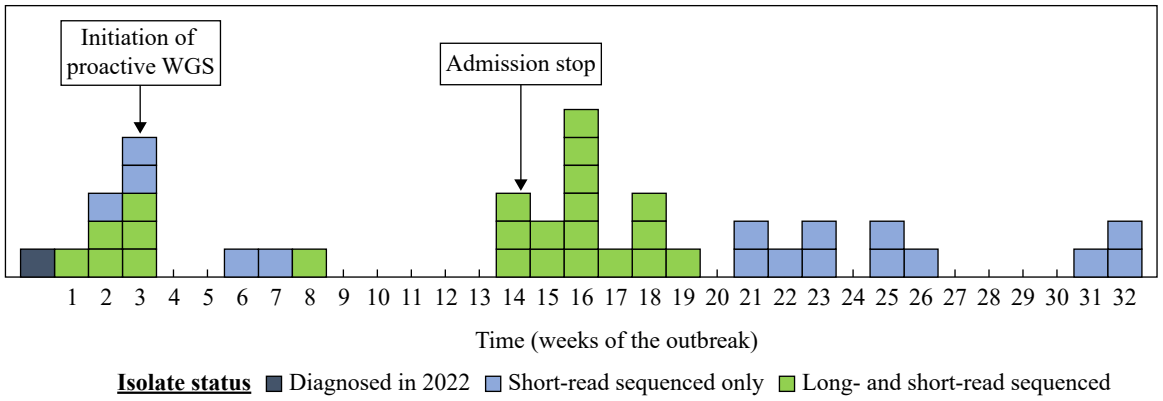

Figure 1. Timeline of nosocomial *Serratia* spp. detections. Nosocomial cases with confirmed genetic relatedness were especially common between weeks 1–3 and 14–19 (green). Week numbers refer to the outbreak timeline. WGS, whole-genome sequencing.

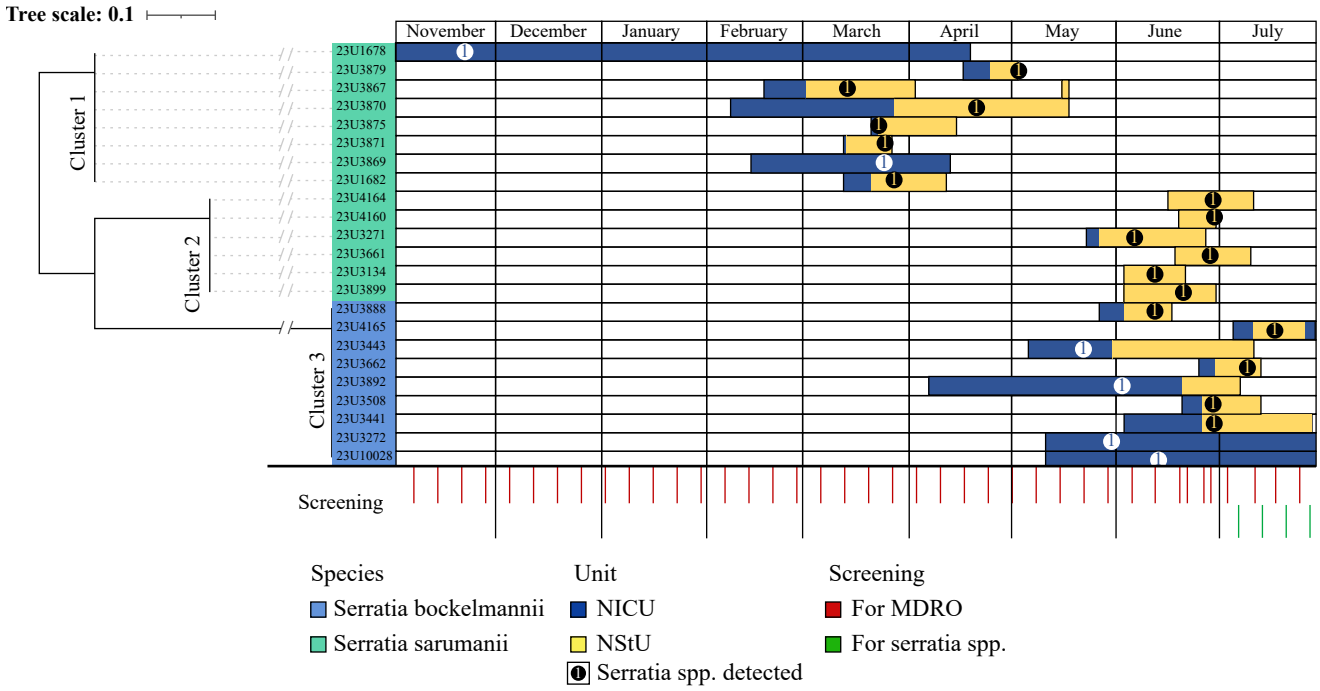

Figure 2. Genome-oriented outbreak line list of the major three clusters. Three large clusters (six to nine cases) were detected through whole-genome sequencing among 40 isolates. Taxonomy analysis revealed that two were due to *Serratia sarumanii* (light green) and to *Serratia bockelmannii* (light blue). The line list reveals that Clusters 1 and 3 were transmitted both at the neonatal intensive care unit (NICU) (dark blue) and the neonatal stepdown unit (NStU) (yellow), whereas transmissions within Cluster 2 only occurred at the NStU.

per week (including an interwave period between weeks 27–30), maintained until week 32 (end of observation).

Microbiological investigations

The swabs were plated on selective agar (MacConkey agar; Biomerieux, Nürtingen, Germany and CHROMagar ESBL, Mast Diagnostic) and cultivated at 37 °C for 24 (MacConkey agar) or 48 h (CHROMagar), respectively. Species identification was performed using MS (Vitek MS Biomerieux acquisition station V1.6.0, Nürtingen, Germany) and antimicrobial resistance (AMR) testing via microdilution technique (Vitek 2, Biomerieux, Nürtingen, Germany).

WGS

Short-read sequencing was performed at University Hospital Regensburg for all available *S. marcescens* (MS-identified) isolates ($N = 40$).

Genomic DNA was extracted using the Macherey–Nagel DNA extraction kit (Macherey–Nagel, Düren, Germany) following the manufacturer's protocol. DNA concentration was measured using a Qubit fluorometer (Life Technologies, Darmstadt, Germany) to ensure sufficient DNA quantity and quality.

Libraries prepared using the Illumina Nextera XT DNA Library Preparation Kit (Illumina Germany, Berlin, Germany) were paired-end sequenced (2 × 150 bp) on an Illumina

NextSeq 550 platform, achieving a minimum coverage of >70. Raw reads were processed for quality assessment using FastQC. *De novo* assembly was conducted using SeqSphere+ (Ridom GmbH, Münster, Germany) with SKESA as the assembly algorithm, and AQUAMIS was used for control of assembly quality and completeness [17].

Long-read sequencing was carried out at CeBiTec (Center for Biotechnology, Bielefeld University), using nanopore sequencing on a Promethion P2 sequencing platform. The ligation sequencing kit with native barcode (SQK-LSK-114 with SQK-NBD114.24) by Oxford Nanopore Technologies (ONT, UK) was used according to the manufacturer's protocol. Long-read data were produced on R10.4.1 flowcells (ONT) and basecalled with GUPPY in super-accuracy mode. The raw data were assembled using FLYE v. 2.9.3-b1797 [18].

Taxonomy and WGS data analysis

The long-read nanopore assemblies of isolates in Clusters 1–3 ($N = 23$) were uploaded to the TYGS platform (https://tygs.dsmz.de) for WGS taxonomic analysis [19,20]. Information on nomenclature, synonymy, and relevant taxonomic literature was provided by TYGS's sister database, the List of Prokaryotic Names with Standing in Nomenclature (LPSN, available at https://lpsn.dsmz.de) [20]. In particular, the isolates *S. sarumanii* K-M0706, *S. bockelmannii* LMG 31535, and *S. nevei* LMG 31536 were used for taxonomy purposes [5,6]. The results were generated by TYGS on 26th July 2025. Single nucleotide polymorphism (SNP) calling and comparative genetic analysis was performed using Snippy (https://github.com/tseemann/snippy).

Taxonomy verification of the remaining isolates ($N = 17$) was carried out using the same procedure, but with short-read assemblies.

AMR and virulence factor (VF) profiles were predicted using abricate (https://github.com/tseemann/abricate) in combination with the databases CARD, NCBI AMRFinderPlus, and VFDB [21–23].

Nomenclature

While the term '*Serratia marcescens* complex' was proposed in literature previously, to our knowledge it has not yet been updated to contain all its novel sub-species (e.g., *S. sarumanii*) [24–26]. Therefore, the nomenclature "*Serratia marcescens* (MS-identified)" was used in the manuscript, and refers to all *Serratia* spp. as identified during outbreak analysis prior to WGS and, which can not be distinguished by using MS (see above); *S. marcescens* (without specification of MS identification), *S. sarumanii*, *S. bockelmannii*, *S. nevei*, *S. ureilytica* and *S. montpellierensis* were used when referring to WGS-confirmed species.

Results

Patient characteristics

Overall, *S. marcescens* was detected by MS in 40 predominantly male ients aged 2–169 days during a period of 17 weeks. Patient characteristics are given in Table I.

Diagnosis was predominantly achieved through screening ($N = 37$), while two patients were initially identified with conjunctivitis prompting analysis, and one experienced a BSI. Seven patients, initially identified through screening, later developed mostly unspecific signs of infections. Notably, conjunctivitis remained infrequent during this period in children negative for *S. marcescens* (MS-identified). One patient (index patient) died due to preterm complications (colitis) unrelated to *S. marcescens*.

Table I
Patient demographics and clinical profiles

Demographics	Cases ($N = 40$)
Female/male, *N*	15/25
Length of stay, days (mean, min–max)	46 (2–169)
Fatal outcome	1
Characteristics of birth	
Preterm birth	32
Birth weight, g (mean, min–max)	2059 (455–10000)
Total gestational age, weeks (mean, min–max)	32 (23–41)
Mode of delivery[a]	
Vaginal	22
Cesarean section	17
Serratia	
Infection	7
First detection after birth/onset, day (mean, min–max)	20 (1–143)
Hospitalization	
Initial ICU treatment	27
Diagnosed with *Serratia* spp. at ICU	14

ICU, intensive care unit.
[a] Data not available for one patient transferred from another hospital.

Characterization of the isolate collection

All 42 *S. marcescens* (MS-identified) isolates were short-read-sequenced successfully and passed quality control. Pairwise comparison based on ad-hoc core-genome multi-locus sequence typing (cgMLST) (2692 alleles) revealed a genetically diverse population of 14 genotypes, including five clusters of two to nine isolates, seven singletons, and two pairs of twins with identical isolates (Figure 3). One environmental isolate (23U3906) was attributed to Cluster 3, whereas the other (23U3907) was closest related (difference of 16 alleles by cgMLST) to a singleton diagnosed 8 weeks later (23U4793). Within the same cluster, isolates differed by 0–2 alleles (Figure 1). This led to the refutation of the initially assumed prolonged outbreak (Figure 3).

The AMR and VF prediction analysis revealed that while all isolates carried *H-NS*, *CRP*, *fliM*, *fliG*, *aac(6′)-Ic*, distinct, genotype-specific patterns of additional genes were present (Figure 3). Clusters 1 and 2 were positive for *mexI*, *oxqB9*, *cheY*, *SRT-1*, whereas Cluster 3 was positive for *mexI*, *oxqB30*, *fliH*, *tet(41)*, *SRT-1*. Among the remaining 17 isolates, further VFs and AMR genes were found, however, infrequently (Figure 3).

Genome-oriented outbreak analysis

The genomically stratified line list of Clusters 1–3 suggests different transmission dynamics per cluster (Figure 2). In detail, the initial case of Cluster 1 was hospitalized at the NICU

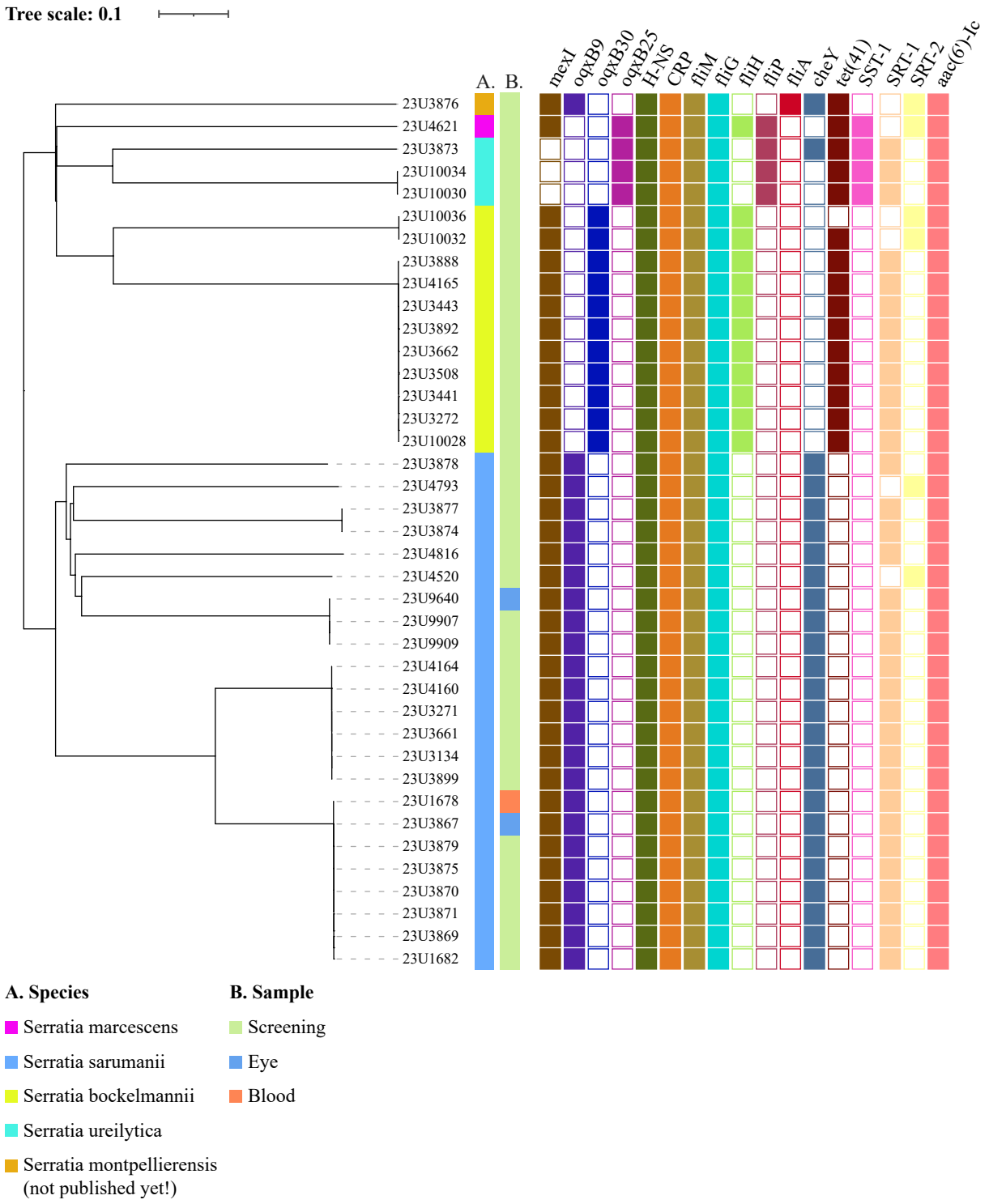

Figure 3. Virulence factor (VF) and antimicrobial resistance (AMR) of *Serratia* spp. In-silico AMR and VF analysis revealed that H-NS, CRP and *aac(6′)-Ic* were present in all isolates. However, several VF and AMR patterns were a cluster and (for Cluster 1–3) species – including with respect to different variants of *fli* (subtypes A, G, H, M, P), *oqxB* (subtypes 9, 25, 30), and SRT-mediated AMR (subtypes 1 to 3). An overview of abbreviations for VF and AMR is available in Supplementary Table S1.

for several months after diagnosis and before further cases were diagnosed. Although all patients ($N = 8$ patients, outbreak weeks 1–3, including two infections: $N_{conjunctivitis} = 1$, $N_{BSI} = 1$) were hospitalized initially at the NICU, cases were diagnosed in similar proportions at both departments. In contrast, all patients of Cluster 2 ($N = 6$) were diagnosed at the NStU during a 4-week-period, and only one patient was briefly hospitalized at the NICU after birth.

Finally, Cluster 3 ($N = 9$, weeks 14–19) comprised patients diagnosed both at the NICU and the NStU. Notably, one environmental isolate collected in week 14 was identical to isolates from this cluster (by cgMLST).

The second environmental isolate was closest related to a singleton, 23U4793, differing by 16 alleles in pairwise comparison by cgMLST.

Taxonomy

Taxonomic identity verification — performed given the non-pigmented isolates and in light of the newly described *S. sarumanii* — assigned patient isolates to five different *Serratia* spp. (Figure 3) [6].

The earliest isolates of Clusters 1 and 2 differed by 36,099 and 36,320 SNP, respectively, from *S. sarumanii* K-M0706, and by 12,694 SNP from each other. Alongside a further nine isolates, they were classified as *S. sarumanii* ($N_{total} = 23$). The earliest isolate of Cluster 3, however, differed by 141,503 SNP from *S. sarumanii* K-M0706. Instead, the closest relative was *S. bockelmannii* LMG 31535 (40,195 SNP), which they were thus reassigned to.

Implications of methylation patterns

Comparing the ad-hoc cgMLST profiles of the samples, obtained by Illumina sequencing, with the corresponding nanopore assemblies revealed differences in some samples when subjected to cgMLST cluster analysis. Interestingly, bioinformatic analysis identified multiple methylation patterns only in samples identified as *S. sarumanii*, but not in *S. bockelmannii* samples. Moreover, this phenomenon cannot be detected in the Illumina sequencing due to the PCR sample processing.

Clinical presentation in light of WGS

Notably, different *Serratia* spp. appeared to be distributed unevenly across patient subgroups (Figure 4). In detail, patients with *S. bockelmannii* (Cluster 3) had immature lungs at birth (4/9), and developed neonatal respiratory distress syndrome (ARDS) (6/9) more frequently than *S. sarumanii* patients (Cluster 1: 2/8 and 3/8, respectively; Cluster 2: 4/6 and 1/6, respectively). Cluster 3, however, had a higher prevalence of congenital heart diseases (Cluster 1: 6/8; Cluster 2: 0/6; Cluster 3: 1/9).

Discussion

In contrast to the predominance of nonfermenters among Gram-negatives in adult ICUs, outbreaks in NICUs are commonly caused by Enterobacterales — in particular *S. marcescens*. While several risk factors predisposing neonates to *Serratia* spp. acquisition have been identified (e.g., neonatal jaundice, non-formula milk feeding, immunological immaturity, VLBW, prolonged hospitalization, empiric antibiotic therapy, indwelling devices or instrumentation), there is still a great knowledge gap on drivers of clinical outbreaks and specific IPC [14,27–30].

Novel, high-resolution typing methods such as WGS have demonstrated their ability to reinterpret outbreak dynamics — until now, however, assuming sole implication of *S. marcescens* [13,14]. Recently, attention was drawn to the sub-population of non-pigmented *S. marcescens* (MS-identified). Molecular analysis divided this subgroup into multiple distinct species, raising important questions regarding possible species-specific IPC requirements and outbreak potential [5,6]. A recent outbreak report and case–control study of *S. bockelmannii* in a French NICU detected an association of this strain with usage of unproperly processed incubators [31]. *This report* documents the first

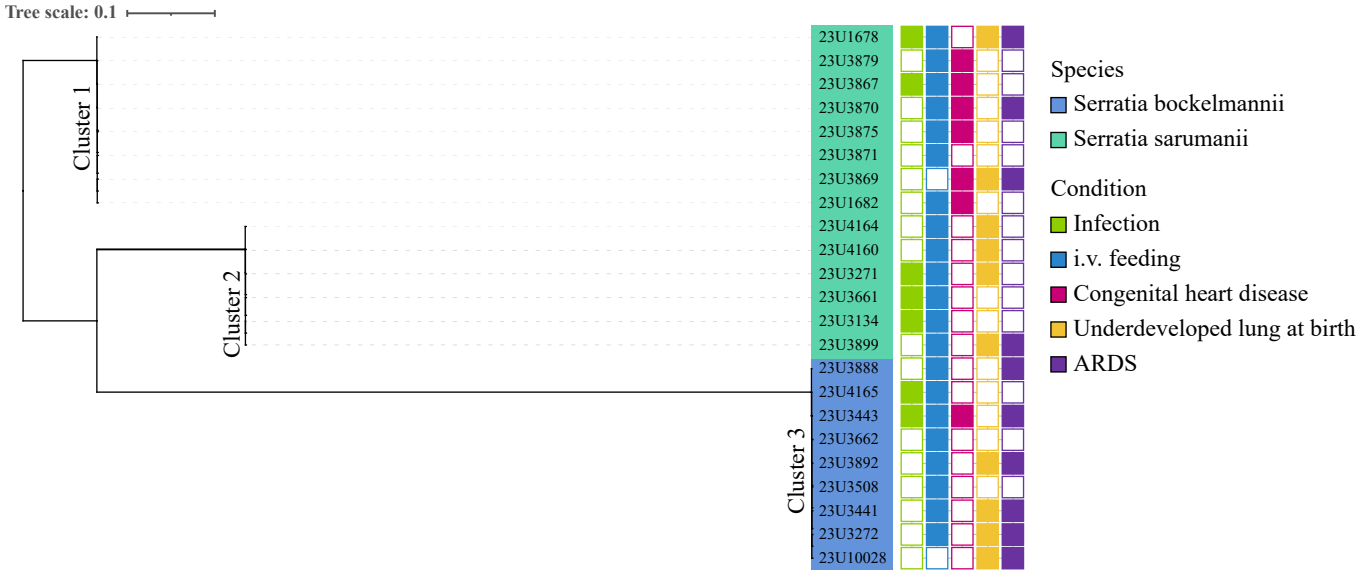

Figure 4. Clinical characteristics of clustering *Serratia sarumanii* and *Serratia bockelmannii* isolates. Clinical analysis indicates that newborns with underdeveloped lungs at birth and acute respiratory distress syndrome (ARDS) were more commonly diagnosed with *S. sarumanii*, whereas congenital heart disease was more common in newborns with *S. bocklemannii*.

concurrent outbreaks of *S. sarumanii* and *S. bockelmannii in a clinical setting. Additionally, it provides the first genome-oriented evidence that outbreaks whose dynamics would formerly have been categorized as complex and interventions as ineffective, could be better explained by either multi-species outbreaks with different environmental characteristics or by multiple, now possibly distinguishable outbreak sources*.

Firstly, the molecular analysis of the putative prolonged outbreak uncovered a polyclonal *Serratia* spp. population comprising 14 genotypes. On species level and in contrast to the initial MS-based identification, only one of 40 isolates was confirmed as *S. marcescens sensu stricto*. The remaining isolates — including three large clusters — were assigned to four other species: *S. sarumanii* ($N = 23$, including Clusters 1 and 2), *S. bockelmannii* ($N = 11$, including Cluster 3 and a pair of twins), *S. ureilytica* ($N = 3$, including a pair of twins) and *S. montpellierensis* ($N = 1$). Notably, this is the first report of *S. montpellierensis* in human samples after its first identification in insects (wasp) [7].

Secondly, the perceived pre-WGS outbreak dynamics included diagnosis in two waves with a gap of 4 weeks, and involvement of two wards of the department to a different extent. This initial inability to distinguish between a perpetuating outbreak and multiple reintroductions of *Serratia* spp. hindered the assessment of intervention effectiveness, ultimately leading to a costly 4-week admission stop to ensure patient safety. WGS, however, concluded a gross overestimation of transmission events through standard diagnostics. Contrary to initial assumptions, only 23 of 40 isolates were outbreak-related, the outbreaks concluded by week 19, and only one of two environmental isolates was cluster-related. Moreover, the 23 outbreak-related cases were split into three distinct strains with separate transmission patterns, possibly suggesting different outbreak drivers. These findings align with growing evidence that suggests low reliability of WGS-free analyses, underscoring the importance of genome-oriented outbreak confirmation and IPC tailoring. For example, a Belgian study reports eight transmission clusters among 61 patients diagnosed within 18 months, whereas isolates from 17 patients were grouped into four genetic clusters in the Netherlands [10,13].

Thirdly, in analogy to further outbreak reports with negative sampling, source control through decontamination was successful, although we only detected the outbreak source for one strain [12]. Unlike previous reports that detected multiple, strain-specific sources during polyclonal outbreaks, our second environmental isolate only loosely matched (differed in 16 alleles) a singleton from the remaining cohort, which was diagnosed 8 weeks after sink removal [9,13].

Fourthly, computational challenges and an urgent need for building proper WGS implementation expertise among IPC experts is necessary. Here, genome-oriented outbreak analysis was initiated early (week 3), but results were delayed due to an apparently increased rate of sequencing errors. Bioinformatics analysis revealed that data analysis was complicated by high levels of methylation among *S. sarumanii* isolates. Similar experiences were reported in *S. marcescens* before the delimitation of this novel species, leaving it unclear whether this observation is species-specific, but underscoring possible association with outbreak potential [32]. Beyond computational challenges, however, recognition of these methylation levels in these novel species could be important for future IPC. Methylation plays an important role in bacteriophage defense, VF expression and pathogenesis, possibly driving its clinical relevance [33,34]. The hypothesis is reinforced by the distinct VF and AMR profiles shown in Figure 3, possibly suggesting evolutionary gene conservation.

Despite limitations due to low case numbers, we also observed variation in the occurrence of certain strains in patient sub-groups with certain clinical conditions: *S. sarumanii* (notably in Cluster 2) predominated among patients with congenital heart disease, whereas *S. bockelmannii* was more frequent in patients with ARDS. Similarly, Sansom *et al*. showed strain variation by sample site (respiratory vs conjunctival) [15]. Further, as in our report, male patients were involved more frequently in an outbreak of *S. bockelmannii* reported from France [31]. However, the implications of these observations remain unclear as statistical testing was not carried out due to the small sample size. It is unclear whether this reflects true predilections or undetected common sources. Behavioural factors (e.g., hand hygiene compliance), organizational structures (e.g., fixed staff-patient assignments), and the need to train inexperienced parents in NICU care may strongly influence transmission pathways and confound interpretation [8,11,13].

In conclusion, although genome-oriented analyses have greatly improved modern IPC — here for *Serratia* spp. — many questions remain unanswered including questions related to the following: (1) the impact of proper species identification on our understanding of pathogen outbreak dynamics, (2) the establishment of validated species-specific cut-off values suitable for pairwise comparison and outbreak analysis, (3) species-specific differences transmission pathway or probability, and (4) the development of specialized WGS expertise for routine, targeted IPC alongside the development of comprehensive data analysis tools [13]. Ultimately, such data could facilitate the development of effective, evidence-based, resource-optimized IPC that shapes a healthy clinical microbiome surrounding vulnerable patients with a low gestational age at birth, VLBW, and prolonged hospitalization — as observed in our cohort. This would positively impact patient health long term, due to known dependency on the microbiome [35].

CRediT authorship contribution statement

A. Rath: Writing — original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **L.J. Klages:** Writing — original draft, Investigation, Formal analysis. **B. Kieninger:** Investigation, Formal analysis, Data curation. **A. Eichner:** Methodology, Investigation. **A. Keller-Wackerbauer:** Investigation. **S.M. Wellmann:** Writing — review & editing, Resources. **A. Ambrosch:** Writing — original draft, Investigation. **J. Fritsch:** Investigation. **M. Kabesch:** Writing — review & editing, Resources. **C. Rückert-Reed:** Investigation, Formal analysis. **T. Busche:** Investigation, Formal analysis. **J. Kalinowski:** Writing — review & editing, Resources. **W. Schneider-Brachert:** Writing — review & editing, Supervision, Resources, Conceptualization.

Ethics statement

The project was approved by the Ethics Committee of the University Hospital of Regensburg within the decision with identification number 25-4311-104.

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Conflict of interest statement

All authors declare that they have no conflict of interest regarding the content of this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2025.12.008.

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