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BRIEF REPORT

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Application of next-generation sequencing to investigate *a Serratia marcescens* outbreak in a neonatal intensive care unit

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Abstract

Introduction Nosocomial infections in Neonatal Intensive Care Units (NICU) are a major concern due to the vulnerability of premature and immunocompromised infants. *Serratia marcescens* is an opportunistic pathogen often involved in these infections, contributing significantly to morbidity and mortality. Integrating Next-Generation Sequencing (NGS) into infection control programs can enhance detection, surveillance, and prevention efforts. This study aimed to develop a mapping-based pipeline for strain typing and phylogenetic analysis of nosocomial infections, enabling detailed comparison of microbial genomes.

Methods A retrospective study describing the outbreak was conducted on 18 *S. marcescens* strains from 14 patients and 2 from environmental swabs collected in the NICU of the University Hospital of Udine between 2023 and 2024. Genomic DNA was extracted and libraries were prepared using the FX DNA Library Preparation Kit (Qiagen). Whole Genome Sequencing (WGS) was performed using an Illumina MiSeq platform (2 × 300 bp paired-end). Data analysis was carried out with CLC Genomic Workbench (Qiagen), using a custom-optimized pipeline for sequence typing (ST). The bioinformatics workflow was developed and validated in-house to ensure accurate SNP-based phylogenetic analysis.

Results WGS revealed phylogenetic relationships among strains. Six isolates showed close genetic relatedness. Identical genotypes were detected in strains from patient blood samples, rectal swabs, and environmental sources, suggesting potential transmission links.

Conclusions NGS offers detailed insights into the molecular epidemiology of infections and colonization in the NICU. The genomic data generated can support real-time, evidence-based refinement of infection control strategies, contributing to improved patient safety and outbreak prevention.

Keywords Neonatal intensive care unit (NICU), Nosocomial infection, Serratia marcescens, Health surveillance, Whole genome sequencing (WGS), Next generation sequencing (NGS), Phylogenetic tree

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Introduction

Nosocomial infections, or hospital-acquired infections, present an arduous challenge within Neonatal Intensive Care Units (NICU), where the patient population is exceptionally vulnerable. These patients, primarily premature and immunocompromised infants, are at an increased risk due to their underdeveloped immune system and the necessity for invasive medical procedures*(*Gastmeier et al., [7, 11, 21]. Among the various pathogens responsible for nosocomial infections, *Serratia marcescens* stands out as a significant opportunistic bacterium. This microorganism is frequently implicated in outbreaks within NICU, contributing substantially to morbidity and mortality rates. *Serratia marcescens*, a member of the Enterobacterales family, exhibits remarkable adaptability and resistance to multiple antibiotics, making it a formidable pathogen in clinical settings *(*Assadian et al., [2, 15, 23]. The ability of this bacterium to thrive in moist environments, including medical equipment and solutions, exacerbates its potential for causing widespread infections. Given its resilience and the serious implications of its presence in NICU, there is an urgent need for advanced methods to detect, monitor, and control infections caused by *Serratia marcescens* and other nosocomial pathogens [9, 23]. The pathogen's resistance to multiple antibiotics necessitates advanced infection control measures and underscores the importance of genomic surveillance for effective management *(*Assadian et al., [2, 5, 17].

Current surveillance methodologies in NICU primarily rely on traditional microbiological techniques, including culture-based methods and phenotypic assays, to identify and track pathogens. These methods, while essential, have several limitations *(*Assadian et al., [2, 14, 23]. Culture-based techniques can be time-consuming, often requiring several days to yield results, which delays the implementation of appropriate infection control measures. Additionally, these methods may not detect all pathogens, especially those present in low abundance or those that are difficult to culture. Phenotypic assays, although useful for initial identification, lack the resolution needed to distinguish between closely related strains of the same species, making it challenging to accurately track the source and transmission routes of infections *(*Duggan et al., [5, 10, 11, 20].

The integration of Next-Generation Sequencing (NGS) technologies into infection control programs offers a promising avenue for addressing these challenges *(*Mao et al., [14, 16]*).* NGS provides comprehensive genomic data, enabling precise identification and characterization of bacterial strains. This level of detail is crucial for understanding the transmission dynamics and evolutionary trajectories of pathogens within healthcare settings *(*Jiaet al., [10, 13].

In recent years, NGS has revolutionized the field of microbial genomics, offering innovative insights into the genetic makeup and evolution of pathogens. This technology facilitates the high-resolution analysis of entire bacterial genomes, allowing for the detection of subtle genetic variations that may contribute to virulence, antibiotic resistance, and transmission. Furthermore, the ability to perform whole genome sequencing (WGS) in real-time supports the timely identification of infection outbreaks and the implementation of targeted interventions *(*Kurtz et al., [12, 14, 16, 20]. Additionally, understanding the genetic diversity and evolutionary history of pathogens like *Serratia marcescens* can shed light on the mechanisms driving their adaptation and persistence in hospital settings. This knowledge is critical for developing novel therapeutic strategies and improving the efficacy of existing treatments *(*Gentile et al., [8, 9, 14]. By integrating genomic data with clinical and epidemiological evidence, researchers and healthcare providers can gain a holistic view of infection dynamics, leading to more informed decision-making and better patient care *(*Howard-Jones et al., [9, 10, 12, 19].

The primary objective of this study was to develop a robust, mapping-based pipeline for creating phylogenetic trees from bacterial whole genome sequences (WGS). This pipeline facilitates the typing of nosocomial infection strains, allowing for detailed evolutionary analyses and comparative studies of microbial genomes. Our pipeline has been applied to multiple clinically relevant bacterial pathogens, but here we focus on a *Serratia marcescens* cluster in a NICU due to its particular clinical and environmental significance.

Through the implementation of this advanced genomic approach, the study aimed to contribute to the broader effort of reducing the incidence and impact of nosocomial infections in neonatal care environments.

This study underscored the potential of NGS technologies to transform infection control practices, offering a powerful tool for safeguarding the health of the most vulnerable patient populations.

Materials and methods

Samples

For this retrospective study, *Serratia marcescens* isolates were collected between 2023 and 2024 from various clinical and environmental sites of the Neonatal Intensive Care Unit (NICU) of the University Hospital of Udine. A focal outbreak was identified when *S. marcescens*–positive patients appeared within a short time frame. Colonized infants were detected through routine surveillance swabs, which allowed early identification of cases in asymptomatic patients and triggered the outbreak investigation. During the surveillance period, a total of 14 patient samples and 2 environmental swabs were

collected and tested in the NICU. Of the 14 patients who tested positive for *Serratia marcescens*, 2 developed a clinical infection, while the remaining 12 were colonized without any signs of active disease. In particular, the 18 isolates from 14 patients, comprising 2 blood samples, 7 rectal swabs, 3 oropharyngeal swabs, 5 pharyngeal swab/rectal swab and 1 central venous catheter (CVC) sample, as well as 2 isolates from 2 environmental swabs, totaling 20 isolates/rectal swab and 1 central venous catheter (CVC) sample, as well as 2 environmental swabs, totaling 20 strains. The inclusion of colonized cases allowed us to track the early circulation of the strain, offering a clearer picture of how the microorganism spread over time. The samples we used for our analysis were obtained as cryostocks of the aforementioned isolates, streaked onto standard blood agar plates, and incubated at 37 °C to obtain single colony.

Primary cultures were first isolated from patient or environmental samples by an independent external microbiology laboratory, which performed morphological, phenotypic, and biochemical characterization. Selected colonies were obtained by culturing the samples on MacConkey agar plates for 18–24 h at 37 °C in a 5% CO_2 atmosphere. The laboratory then prepared cryostocks from these colonies, which were subsequently revived in tryptic soy broth and grown overnight prior to DNA extraction and sequencing.

The study was approved by the local institutional review board (*IRB:338/2024 Tit III cl 13 fasc.209/2024*).

DNA isolation

Genomic DNA was isolated using the Quick-DNA Fungal/Bacterial Kit (Zymo Research), supplementing the lysis buffer with lysozyme. The DNA concentration and quality were determined using a NanoDrop 2000 spectrophotometer and a Qubit Flex Fluorometer (Thermo Fisher Scientific).

Library Preparation and sequencing

Library preparation was conducted staring from 300ng of genomic DNA using the FX DNA Library Preparation Kit (Qiagen) and the NexteraTM XT Index kit (Illumina), following manufacturer's instruction. Whole Genome Sequencing (WGS) was performed on an Illumina MiSeq System using a 2 × 300 bp paired-end run.

Data analysis

Data analysis Sequencing files were analyzed with CLC Genomic Workbench launching a home-made design pipeline. The computational pipeline for SNP tree construction begins with data input, where raw sequencing data is collected and preprocessed for quality control. The next step involves aligning the reads to a reference genome, followed by variant calling, where Single Nucleotide Polymorphisms (SNP) are identified through comparison with the reference sequence. After variant calling, SNPs are filtered based on quality metrics to eliminate low-confidence calls, ensuring high accuracy. The filtered SNP are then annotated, if needed, to provide functional or positional context. Once annotated, the SNP are compiled into a matrix format compatible with phylogenetic analysis tools. This matrix is used to construct the SNP tree, applying appropriate algorithms to infer evolutionary relationships. The resulting tree is then visualized and interpreted, allowing insights into genetic variations and phylogenetic patterns. To compute the phylogenetic distance between samples, a distance matrix was derived from branch lengths of the phylogenetic tree. Samples were then clustered using Euclidean distance and the *complete* linkage method implemented in the pheatmap R package (*Kolde R (2019). _pheatmap: Pretty Heatmaps_. R package version 1.0.12*, <https://CRAN.R-project.org/package=pheatmap%3E.*).* Phylogenetic trees were generated and visualized in R using the ape package, where root-to-tip distances were normalized and converted into percentage similarity values annotated on nodes and tips to highlight relative divergence *(Paradis E, Schliep K. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics. 2019 Feb 1;35(3):526–528. doi*: https://doi.org/10.1093/bioinformatics/bty633. *PMID: 30016406.).*

Results

Clinical characteristics

A total of 14 infants, admitted into NICU between 2023 and 2024, were included in this retrospective study. Among the outbreak cases, clinical infections were observed as confirmed sepsis, with positive blood cultures, while colonized patients were identified through routine surveillance swabs. The the mean age at first positive sample was 15,3 days; no patients died. A infection control measures bundle was immediately implemented to minimize further nosocomial spread of the pathogen. In particular, the infection control bundle implemented during the outbreak included standard precautions such as hand hygiene, use of personal protective equipment, environmental cleaning and disinfection. Hand hygiene in the NICU plays a critical role as an infection prevention and control (IPC) measure, influencing transmission pathways and acquisition dynamics, including patient-to-patient and environment-to-patient spread.

Diagnostic specimens collected from patients comprised 2 blood samples, 9 rectal swabs, 2 oropharyngeal swabs, and 1 central venous catheter (CVC). Furthermore, other 2 strains isolated from environmental swab, specifically from the sink drain tubes, were included in the phylogenetic analysis. This is a retrospective study, and the samples were collected over the course of one

year. The increasing patient number indicates the chronological order of collection, with Patient 1 being the earliest and Patient 14 being the latest. Environmental swabs were collected one year after Patient 1, around the time of Patient 13 and 14. The duplicate samples from the same patient were collected one week apart. All details available regarding our case-study are reported in Table 1. *Serratia marcescens* strains enriched and characterized with classical microbiological methods were morphologically and phenotypically identical. Unfortunately, we do not have access to detailed phenotypic data or antibiotic susceptibility profiles because the isolates were provided anonymously, with only the information reported in Table 1. Therefore, we cannot assess whether the isolates were all sensitive or exhibited different resistance phenotypes. For these reasons, to assess their correlation, genomic analysis was necessary to compare the molecular signature of each isolate.

Table 1 Characteristics of patients included in this study

Patient ID	Age (Days)	Number of Samples	Type of SampleS	Specie
Patient 1	36	2	Central Venous Catheter (CVC)/blood	*Serratia marcescens*
Patient 2	23	1	rectal swab	*Serratia marcescens*
Patient 3	11	2	pharyngeal swab	*Serratia marcescens*
Patient 4	42	2	rectal swab	*Serratia marcescens*
Patient 5	12	2	rectal swab	*Serratia marcescens*
Patient 6	4	1	rectal swab	*Serratia marcescens*
Patient 7	11	1	blood	*Serratia marcescens*
Patient 8	8	1	pharyngeal swab/rectal swab	*Serratia marcescens*
Patient 9	8	1	pharyngeal swab/rectal swab	*Serratia marcescens*
Patient 10	7	1	pharyngeal swab/rectal swab	*Serratia marcescens*
Patient 11	6	1	rectal swab	*Serratia marcescens*
Patient 12	16	1	pharyngeal swab/rectal swab	*Serratia marcescens*
Patient 13	10	1	pharyngeal swab/rectal swab	*Serratia marcescens*
Patient 14	8	1	pharyngeal swab	*Serratia marcescens*
Environmental swab 1		1		*Serratia marcescens*
Environmental swab 2		1		*Serratia marcescens*

Phylogenetic analysis

The sequences were analyzed using a typing approach that compared variants among the reference sequence and isolates. All patients yielded *Serratia marcescens* isolates, confirmed through taxonomic profiling. Phylogenetic analysis based on whole genome sequences revealed that these strains clustered into two major groups (A and B), originating from a reference ancestral strain (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_030291735.1/) classified in the NCBI RefSeq Database. Evolutionary analyses of strain mutations were conducted with respect to the repository reference sequence to understand the genetic variations (SNP) and evolutionary relationships among the strains. This analysis allowed us to identify mutations that have occurred over time among our *Serratia marcescens* isolates and to examine how these changes differentiate the strains from each other. By tracking these variations, we were able to infer the evolutionary relationships and dynamics within the cluster, providing insights into potential transmission patterns as well as implications for pathogenicity and resistance. Furthermore, by comparing variants across all isolates, a distance matrix was generated to highlight the genomic similarities and differences among the strains in our study (Supplementary File 1 and Supplementary Fig. 1).

Then, a phylogenetic tree was constructed using mutational patterns enriched of all 20 isolates of *Serratia marcescens*. Figure 1, depicted their evolution and the distances from more ancestral sequence and the other global isolates. The greatest genetic distance was observed in a cluster of 5 strains isolated from Patient 7, Patient 6, Patient 14 and Patient 3 and 1 environmental swab. Another cluster of strains consisted of 4 patients (Patient 1, Patient 2, Patient 4 and 5) and another environmental swab. This latter cluster showed more similarity to the ancestral reference sequence and less evolutionary distance with respect to the other cluster. It could be hypothesized for both clusters that an initial transmission between the environment and patients occurred, which then spread to other infants present in the NICU during that time period.

Considering the mutational differences accumulated among the strains, it can be hypothesized that the cluster with the highest number of mutations originated from the smaller, less evolved cluster (closer to the ancestral sequence). This suggests that the strain acquired a higher selective advantage. Notably, some of these mutations have been identified in antimicrobial resistance (AMR) genes, see Fig. 2; Table 2. The sunburst chart illustrating the distribution of identified genetic elements related to antimicrobial resistance (AMR). The chart categorizes the genes based on their mechanism of resistance, which includes efflux pump complexes, antibiotic inactivation,

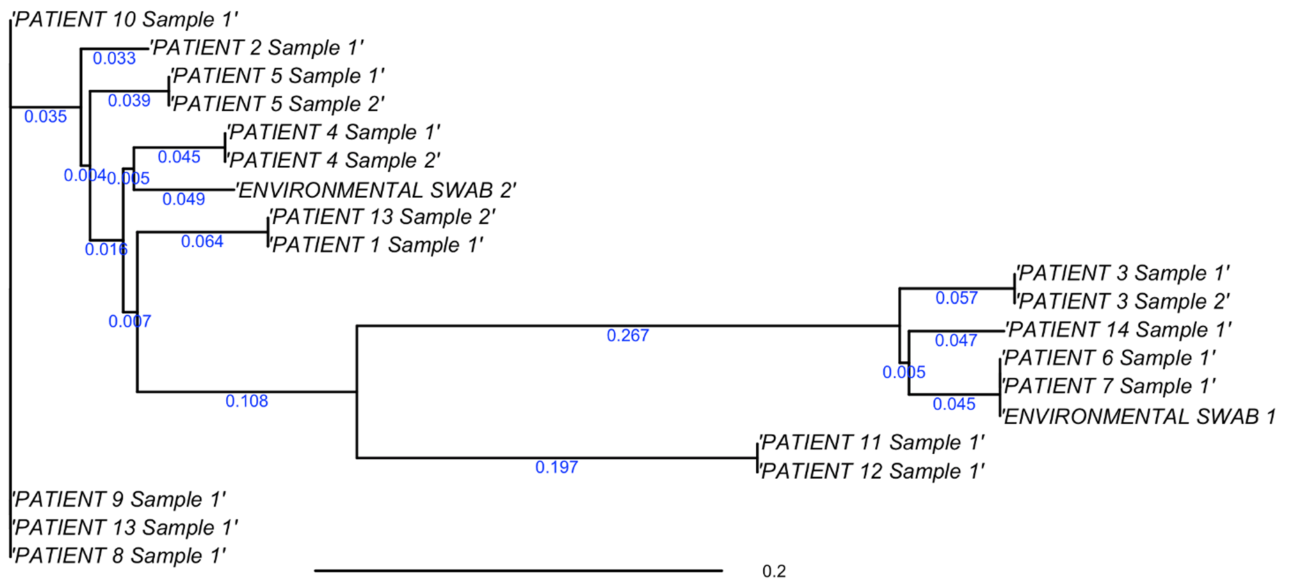

Fig. 1 Phylogenetic tree based on SNP differences among patient samples and environmental swabs. Branch lengths represent genetic distances, with values indicated in blue. The tree highlights clusters of closely related samples, reflecting potential transmission links or shared sources of contamination

Fig. 2 Merged resistance table showing the classification of AMR peptide markers found in sequenced strains of *Serratia marcescens*

Table 2 List of antimicrobial resistance (AMR) genes and regulatory elements identified in the analyzed *Serratia marcescens* isolates. The table reports the resistance mechanism and a brief description of each gene's function, including efflux pumps, antibiotic inactivation enzymes, target protection proteins, and regulatory elements potentially involved in resistance

Gene / Element	Mechanism of Resistance	Description / Function
acrB	Efflux Pump Complex	Part of the AcrAB-TolC efflux system, responsible for multidrug resistance.
acrA	Efflux Pump Complex	Component of the AcrAB-TolC efflux pump, working with AcrB to expel antibiotics.
mdtB	Efflux Pump Complex	Multidrug efflux pump associated with resistance to various antibiotics.
msbA	Efflux Pump Complex	An ATP-binding cassette transporter involved in lipid transport and drug resistance.
oqxB	Efflux Pump Complex	Part of the OqxAB efflux pump, conferring resistance to fluoroquinolones.
AAC(6')-Ic	Antibiotic Inactivation	Aminoglycoside acetyltransferase, modifying aminoglycosides to confer resistance.
QnrB58	Antibiotic Target Protection	Plasmid-mediated protein that protects DNA gyrase from fluoroquinolone action.
SRT-1	Antibiotic Target Protection	Potentially associated with modification or protection of antibiotic targets.
tet(41)	Tetracycline Resistance	Confers resistance to tetracyclines through efflux or ribosomal protection.
baeR	Transcriptional Regulation	Part of a two-component system regulating efflux pumps and stress response.
rosB	Regulatory Protein	Likely involved in transcriptional regulation of resistance mechanisms.
CRP	Regulatory Protein	cAMP receptor protein, which may regulate various metabolic and resistance genes.

antibiotic target protection, transcriptional regulation, and tetracycline resistance. The most prominent category detected is the efflux pump complex, indicating its major role in the observed resistance profiles. Additionally, genes involved in antibiotic inactivation and target protection were identified, suggesting various mechanisms contributing to antimicrobial resistance. Notably, the genes *acrB*, *acrA*, and *mdtB* associated with the AcrAB-TolC efflux pump system are the most represented, emphasizing the importance of efflux mechanisms in the resistance observed. The presence of *QnrB58* and *AAC(6')-Ic* also indicates resistance mechanisms targeting fluoroquinolones and aminoglycosides, respectively. The identification of AMR genes was used to assess which genomic regions the SNP used for the phylogenetic tree fell into. The analysis is purely descriptive and not intended to explain the outbreak dynamics.

Isolates from the same patient have proven to be genetically identical. The anatomical site does not seem to select for different strains; rather, the genetic differences observed between different patients appear to result from antibiotic treatments and the development of mutations in AMR genes, as well as from an interaction between the patient's and pathogen's genomes.

Discussion

In this study we report a case series of 14 hospitalized neonates with nosocomial *Serratia marcescens* infections and colonization in the NICU of our hospital. Of the 14 neonates who tested positive for *Serratia marcescens*, 2 developed clinical infection while 12 were colonized without symptoms. Both groups were included in the transmission analysis, as colonized patients play a crucial role in spreading opportunistic pathogens in the NICU. Asymptomatic carriers can act as silent reservoirs, contributing to bacterial dissemination and posing a risk to other vulnerable neonates. Including colonized cases allowed for a more accurate reconstruction of transmission dynamics, early strain circulation, and potential super-spreaders, which is essential when using whole-genome sequencing for outbreak investigations.

Traditional microbiological methods are essential but limited in outbreak tracking due to their lower resolution and speed. In contrast, Whole Genome Sequencing (WGS) provides a high-resolution analysis, allowing precise strain differentiation, accurate transmission tracking, and identification of antimicrobial resistance and virulence factors. By implementing a NGS pipeline for strain typing, the outbreak of *Serratia marcescens* was effectively traced by discovering a possible transmission model. 5 strains isolated from 4 patients, and 1 environmental swab were found to belong to a major cluster based on molecular phylogenetic analysis. A second cluster composed by 4 patients' isolates emerged but more similar to the ancestral reference sequence and less evolutionary distance from the main cluster.

Our pipeline for SNP tree construction follows a structured workflow where raw sequencing data undergoes quality control, alignment to a reference genome, and variant calling to identify SNPs. High-quality SNPs are filtered, optionally annotated, and compiled into a matrix for phylogenetic analysis, enabling accurate tree construction and visualization of evolutionary relationships.

Several pipelines have been developed to optimize the use of WGS in pathogen tracking. For instance, BacPipe is an automated, user-friendly pipeline designed for rapid analysis of bacterial whole-genome sequencing data, streamlining processes from raw reads to comprehensive reports. Another example is DODGE (Dynamic Outbreak Detection for Genomic Epidemiology), an algorithm that dynamically selects and compares genetic

thresholds to enhance outbreak detection accuracy [4, 18, 22]. These tools exemplify the advancements in bioinformatics that complement WGS, providing robust frameworks for real-time surveillance and effective outbreak response.

Mutations acquired by the most evolved cluster suggested a possible derivation from the ancestral one and from the other tested isolates, representative of a higher selective advantage. In fact, *Serratia marcescens*, like other bacteria, undergoes genetic mutations that can be spontaneous or induced by environmental pressures. These mutations occur in various genes and can lead to changes in the bacterium's physiology and behavior*(*Anagnostakis et al., [1, 3, 15]. Key mutations often involve genes responsible for essential cellular processes, virulence factors, and survival mechanisms. A significant aspect of the evolution is the development of antimicrobial resistance. Selective pressures, such as the widespread use of antibiotics, drive the evolution of *Serratia marcescens.* Strains with advantageous mutations survive and proliferate, while those without such mutations are eliminated. This process, known as selective evolution, results in the predominance of AMR strains *(*Gastmeier et al., [7, 14, 15, 19]. In our study, the most represented genes were *acrB*, *acrA*, and *mdtB* from the AcrAB-TolC efflux system, along with *QnrB58* and AAC(6')-Ic, which are associated with resistance to fluoroquinolones and aminoglycosides.

By analyzing SNV and comparing them to an ancestral reference sequence, researchers could hypothesize evolutionary pathways.

The close quarters and often compromised health of NICU patients facilitate the spread of *Serratia marcescens* through direct contact or contaminated surfaces*(*Anagnostakis et al., [1, 23]. In our outbreak, 14 infants developed infections or colonization after being housed in the same room, likely through environmental contamination or indirect contact with healthcare workers.

Our study has inherent limitations due to its retrospective design and single-center setting. These factors may affect the generalizability of our findings and should be considered when interpreting the results.Another limitation is that our transmission model is based solely on genomic data due to the limited patient information available from anonymized samples. Future studies incorporating classical epidemiological data, such as room contacts and overlapping stays, could further validate and refine the model.

An unresolved question remains how does *Serratia marcescens* enter the NICU. After sanitization, environmental swabs consistently give negative results, yet *Serratia* infections frequently reappear. One hypothesis is that the neonates themselves may be transferring the bacteria into the NICU from the delivery room, but this remains speculative. Scientific literature supports the idea that *Serratia marcescens* can persist in healthcare environments despite rigorous cleaning. Its ability to survive on dry surfaces, as well as in aqueous environments, contributes to its persistence. There have been documented cases where *Serratia* strains were transmitted through contaminated medical equipment or fluids, even after disinfection [1]; Cristina et al., [3, 11, 21, 23]. Furthermore, colonization in the maternal vaginal flora or the hospital plumbing system has been identified as a potential source of introduction into NICU. Studies also suggest that asymptomatic colonization in neonates may occur, with bacteria carried from the delivery room or maternal body. These neonates can act as reservoirs, leading to secondary infections within the NICU*(*Anagnostakis et al., [1, 2, 5, 6]*)*. This is particularly concerning due to *Serratia's* ability to evade traditional sanitization methods and form biofilms, making eradication difficult *(*Assadian et al., [2, 5, 7, 15]. Thus, while the hypothesis of neonates introducing the bacteria into the NICU is plausible, further investigation is required to fully understand its transmission pathways.

Furthermore, the sensitivity and rapid results provided by NGS technologies help in identifying infections early and stopping them before they spread*(*Howard-Jones et al., [9, 10, 13]. Prompt diagnosis and isolation of affected neonates are critical for controlling the diffusion of *Serratia marcescens.*

The designed approach aligns with strategies used in controlling other nosocomial pathogens, which also relies on early isolation and thorough disinfection.

Monitoring of bacterial shedding and adherence to infection control protocols is essential for preventing future outbreaks.

Conclusion

In conclusion, the successful results obtained from the genomic analysis of *Serratia marcescens* strains allowed for the determination of phylogenetic relationships among the samples and helped us in hypothesize a transmission model outbreak in our NICU.

Clinicians should maintain a high level of vigilance concerning the risk of nosocomial *Serratia marcescens* infections in NICU patients. Prompt implementation of comprehensive infection control measures is crucial for containing outbreaks. In our retrospective case series, the NGS strategies effectively helped in tracing the source of the *Serratia marcescens* infection and its outbreak.

Our study demonstrated the feasibility of implementing NGS for tracking *Serratia marcescens* transmission, providing a level of resolution not achievable with conventional culture-based methods and phenotypic analysis. These results support the integration of NGS into

infection control strategies as a powerful tool for outbreak investigation and transmission mapping, particularly in high-risk settings such as Neonatal Intensive Care Units.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Author contributions

M.B. and N.G. conceptualization, methodology data analysis and curation; B.K., G.V., S.M. and C.L. methodology; C.P. R.C., B.M., G.V., A.F. C.C. and M.B. methodology and interpretation of results; M.B, C.P., R.C. and F.C. writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Data availability

The authors confirm that all relevant data are included in the articleand materials are available on request. Sequencing data have been deposited in the NCBI Short Read Archive (SRA) under BioProject ID PRJNA1235258 and are available at the following link: https://dataview.ncbi.nlm.nih.gov/object/PRJNA1235258?reviewer=b3oun6irfab7g035iq2brbm59o.

Declarations

Competing interests

The authors declare no competing interests.

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