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Major Article

Serratia marcescens outbreak in a neonatology unit of a Spanish tertiary hospital: Risk factors and control measures

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A B S T R A C T

Background: We describe the investigation undertaken and the measures adopted to control a *Serratia marcescens* outbreak in the neonatology unit of La Paz University Hospital in Madrid, Spain.
Methods: Weekly rectal and pharyngeal screenings for *S marcescens* were performed in the neonates starting after detection of the outbreak. Environmental samples and samples from health care workers (HCWs) were obtained for microbiological analysis. An unmatched case-control study was carried out to investigate risk factors for infection/colonization.
Results: The outbreak began in June 2016 and ended in March 2017, affecting a total of 59 neonates. Twenty-five (42.37%) neonates sustained an infection, most frequently conjunctivitis and sepsis. Multivariate logistic regression identified the following risk factors: parenteral nutrition (odds ratio [OR], 103.4; 95% confidence interval [CI], 11.9-894.8), history of previous radiography (OR, 15.3; 95% CI, 2.4-95.6), and prematurity (OR, 5.65; 95% CI, 1.5-21.8). Various measures were adopted to control the outbreak, such as strict contact precautions, daily multidisciplinary team meetings, cohorting, allocation of dedicated staff, unit disinfection, and partial closure. Hands of HCWs were the main suspected mechanism of transmission, based on the inconclusive results of the environmental investigation and the high number of HCWs and procedures performed in the unit.
Conclusions: *S marcescens* spreads easily in neonatology units, mainly in neonatal intensive care units, and is often difficult to control, requiring a multidisciplinary approach. Strict measures, including cohorting and medical attention by exclusive staff, are often needed to get these outbreaks under control.

Serratia marcescens is a gram-negative bacterium from the Enterobacteriaceae family that acts as a ubiquitous pathogen and is able to survive in moist environments, ranging from water and soil to medical devices. *S marcescens* is responsible for outbreaks in neonatal intensive care units (NICUs), causing considerable infections and mortality.[1,2] Preterm neonates are readily colonized because their intestinal microbiota has not yet been established.[3] They are also more susceptible to infection owing to the immaturity of their immune system[4] and have less effective skin and mucosal barriers,[5] which also may be damaged as a result of invasive procedures. The gastrointestinal and respiratory tracts are the primary reservoirs in children, and hospital transmission commonly occurs via passive carriage from the hands of health care workers (HCWs).[6,7] Although the sources of outbreaks are often unclear, some studies have implicated contaminated incubators, soap and hand disinfectants, laryngoscopes,

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breast milk and formula, and parenteral nutrition.[1] Several risk factors have been identified, including low birth weight, mechanical ventilation, invasive procedures, exposure to antibiotics before and after birth, duration of antibiotic therapy, time of hospital admission, history of maternal infection before delivery, surgery, and steroid therapy.[1] *Serratia marcescens* outbreaks are often difficult to control, sometimes persisting for months or even years.

Here we report a large outbreak (59 cases) of *S marcescens* infection occurring in the Neonatal Unit at La Paz University Hospital in Madrid, Spain, between 2016 and 2017. We describe the main characteristics of the outbreak and the measures taken to control it, and identify the main internal and external risk factors involved.

METHODS

Setting

La Paz University Hospital is a 1,300-bed tertiary care university hospital located in an urban area. The architectural and functional characteristics of the Neonatology Unit, which has a total of 73 beds, are as follows: 23 NICU beds, distributed in 3 sections; 24 intermediate care, distributed in 4 pods; and 26 standard care beds, distributed in 4 pods and 5 single rooms for mother-infant care. The sections are not interconnected, and the entry is through a common corridor. The nurse-patient ratio is 1:2 for the NICU and 1:5 for intermediate care and 1:7 for standard care facilities. This Neonatology Unit is a tertiary referral unit with approximately 1,500 admissions per year, one-third of which are in the NICU, with an average length of stay of 13 days. Approximately 15% of the neonates admitted every year are born at <32 weeks of gestational age, 5% are born at <28 weeks, and 10% weigh $<1,500$ g at birth.

The Neonatology Unit includes approximately 230 HCWs, including neonatologists, medical residents, nurses, and nursing assistants. Moreover, owing to the high complexity of cases, many physicians from other specialties and areas of the hospital are frequently asked to examine these patients. Parents are allowed to visit their infants 24 hours a day, and other family members can visit twice a day for 30 minutes accompanied by a parent.

Epidemiologic investigation

As cases emerged, data on the neonates' characteristics and location and date of the first positive sample were collected prospectively by the hospital's Department of Preventive Medicine (infection control team). In addition, the date of admission was recorded to calculate weekly incidence rates. In October 2016, *S marcescens* detection was included in the routine weekly screenings performed in the NICU via rectal and pharyngeal swabs. In December 2016, when several positive clinical samples appeared in non-NICU wards, screening was introduced there as well.

In an effort to control the outbreak, an unmatched case-control study was performed starting in February 2017 to identify external and internal risk factors related to *S marcescens* colonization and infection. Neonates who had been hospitalized in the Neonatology Unit >48 hours were included in the study, considering that all neonates who were admitted in the Neonatology Unit at the same time as cases infected or colonized with *S marcescens* were at risk, based on epidemiologic criteria.

Cases were defined as those neonates with either colonization or infection by *S marcescens*, and controls were defined as those neonates who did not present with either infection or colonization by *S marcescens* and had been screened weekly for carriage while admitted in the unit. Colonization was considered when a rectal or pharyngeal swab or other location culture was positive for *S marcescens* with neither signs nor symptoms of infection. Infection was considered when a culture was positive for *S marcescens* in addition to symptoms or signs of infection based on neonatologists' clinical judgment. Information on the following variables was obtained from the clinical record: sex, age, length of stay before infection/colonization in cases or until discharge in controls (defined as time at risk), and maternal comorbidities during pregnancy, as well as diagnostic and therapeutic procedures and comorbidities in the infants (Table 1). The values of these variables in the cases were always recorded before colonization/infection.

Microbiological methods

The clinical samples were inoculated on different cultured media accordingly with the Microbiology Service protocols. The pharyngeal and rectal surveillance samples were cultured directly on MacConkey agar plates (BD, Heidelberg, Germany). Isolates were identified using a MALDI Biotyper (BrukerDaltonik, Bremen, Germany). Antibiotic susceptibility was determined using the Wider system (Francisco Soria Melguizo, Madrid, Spain) and the MicroScan WalkAway system (Beckman Coulter, Brea, CA), and isolates were categorized as susceptible or resistant according to the European Committee on Antimicrobial Susceptibility Testing. Serial environmental samples were obtained by swabbing the surfaces with a cotton swab previously moistened in sterile saline. Samples were stored in Amies gel transport medium at room temperature until processing. The swabs were inoculated in brain-heart infusion broth (Tec-Laim, Madrid, Spain), vortexed for 30 seconds, incubated overnight at 37°C, and then plated on MacConkey agar. All liquid environmental samples were centrifuged at 3,500 rpm for 10 minutes, after which the precipitate was inoculated in brain-heart infusion broth and MacConkey agar. The genetic relationships between the isolates were determined by automated repetitive-sequence–based polymerase chain reaction using the DiversiLab system (bioMérieux, Marcy l'Etoile, France).[8] The isolates' relatedness was analyzed using the DiversiLab software, version 3.4, which uses the Pearson correlation coefficient to determine distance matrices and the unweighted-pair group method using average linkages to create dendrograms, electropherograms, and virtual gel images.

Environmental investigation

A total of 318 environmental samples were obtained during the outbreak, including medication and nutrition products, medical devices, equipment, ventilation and water systems, and hygiene-related solutions. Two hundred and seventy-eight samples were obtained between October 2016 and February 2017, and 40 more were obtained between March and April 2017, after the case-control study had been performed. Samples from dry surfaces and equipment were obtained using swabs soaked in sterile saline solution. Swabs were wiped across the investigated surface and then introduced in brain-heart infusion broth. To sample sink drains, a long probe/nasogastric tube and a syringe were used to obtain water samples. Air samples were obtained by a volumetric sampler, which sucked 500 L of air onto MacConkey agar plates. The samples obtained are listed in Table 2.

Statistical analysis

Continuous variables were compared with the Student t test or Wilcoxon rank-sum test depending on the normality of the distribution, and categorical variables were explored using the χ^2 test. The potential internal or external risk factors for infection/colonization by *S marcescens* were identified via a case-control study. Risk estimates were calculated using a multivariate forward logistic regression. Variables were introduced in the model if the *P* value was $<.10$ in the

Table 1
Distributions of general and birth-related variables, medical procedures, and comorbidities in cases and controls

	General and birth-related variables											
n = 109	Sex		Delivery		Maternal vaginal-rectal culture*		Corticosteroids during pregnancy		Maternal antibiotherapy before delivery†		Maternal peripartum infection	
	Male, %	Female, %	Vaginal, %	Cesarean, %	Negative, %	Positive, %	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %
Controls	51	49	46	54	54	17	86	14	75	25	95	5
Cases	43	57	63	37	33	9	39	51	67	33	67	33
P value	.450		.079		.770		**< .001**		.410		**< .001**	
	General and birth-related variables											
	Preterm (< 37 wk)		Low weight (< 250 g at birth)		Low weight for gestational age		Weight at birth, g		Gestational age at birth, wk		Time at risk, d‡	
	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %	Mean	SD	Mean	SD	Median	IQR
Controls	60	40	90	10	84	16	2693	1029	36.44	0.50	10	18-5
Cases	17	83	35	65	85	15	1507	134	30.17	0.69	14.5	29-9
P value	**< .001**		**< .001**		.926		**< .001**		**< .001**		**.003**	
	Medical procedures											
	Radiography		Abdominal ultrasound		Brain ultrasound		Cardiac ultrasound		Mechanical ventilation		Invasive ventilation	
	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %
Controls	48	52	43	57	32	68	51	49	43	57	78	22
Cases	4	96	20	80	11	89	22	78	9	91	43	57
P value	**< .001**		**.011**		**.010**		**.002**		**< .001**		**< .001**	
	Medical procedures											
	Noninvasive ventilation		Surgery		Ocular fundus examination		Bladder catheter		Central venous catheter		Nasogastric tube	
	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %
Controls	54	46	87	13	84	16	98	2	67	33	79	21
Cases	15	85	83	17	91	9	98	2	11	89	72	28
P value	**< .001**		.494		.269		.822		**< .001**		.356	
	Medical procedures										Comorbidities	
	Parenteral nutrition		Enteral nutrition		Phototherapy		Transfusions		Electroencephalography		Cardiovascular	
	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %
Controls	75	25	6	94	73	27	83	17	100	0	68	32
Cases	2	98	0	100	50	50	48	52	96	4	41	59
P value	**< .001**		.082		**.014**		**< .001**		.095		**.005**	
	Comorbidities											
	Respiratory		Renal		Urinary		Neurologic		Hematologic		Endocrine	
	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %
Controls	51	49	92	8	95	5	75	25	78	22	84	16
Cases	37	63	80	20	93	7	80	20	39	61	65	35
P value	.151		.073		.691		.474		**< .001**		**.022**	
	Comorbidities											
	Digestive		Previous infection		Previous antibiotherapy		Hyperbilirubinemia		Hyponatremia		Metabolic acidosis	
	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %	No, %	Yes, %
Controls	97	3	76	24	37	63	63	37	100	0	92	8
Cases	76	24	72	28	24	76	43	57	85	15	72	28
P value	**.001**		.599		.145		**.038**		**.001**		**.005**	

NOTE. Bold values are statistically significant (P < .05). *IQR*, interquartile range; SD, standard deviation.
*Vaginal-rectal culture for identification of group B streptococci. Percentages do not sum to 100% because culture was not performed in all pregnant women.
†Antibiotic administration to women with group B streptococci colonization.
‡Length of stay before infection/colonization in cases or until discharge in controls.

Table 2
Environmental samples obtained during the outbreak, before and after closure of the Neonatology Unit

Samples	No.	Result
Samples obtained before closure (October 16 to February 17)		
Drains	5	Negative
Taps	12	Negative
Siphons	31	Positive (n = 3)
Sinks	17	Negative
Water	30	Negative
Chlorhexidine	11	Negative
Hand cream and other moisturizing products	5	Negative
Soap	5	Negative
Hydroalcoholic gel	2	Negative
Milk, breast pumps, and other related devices	9	Negative
Physiological serum	3	Negative
Incubator	45	Negative
Ultrasound and related devices	8	Negative
Air grille and conducts	20	Negative
Room surroundings	20	Negative
Other medical devices	19	Negative
Medication products	13	Negative
Glove boxes	4	Negative
Nasogastric tube	1	Negative
Laryngoscope	2	Negative
Water fountains and coffee machine	3	Negative
Respiratory devices	11	Negative
Phones	2	Negative
Total	278	
Samples obtained after reopening (March-April 17)		
Chlorhexidine	3	Negative
Respiratory devices	3	Negative
Parenteral nutrition in Neonatal Unit		
Solution	10	Negative
Bottles and container bags	7	Negative
Infusion pump buttons	3	Negative
Pump reservoir	2	Negative
Preparation countertop, needle, and heparin	4	Negative
Parenteral nutrition in hospital pharmacy		
Solution	5	Negative
Filtered and nonfiltered water	3	Negative
Total	40	

univariate analysis and were kept in the model when the *P* value was < .05. Statistical analyses were performed using Statistical Package for the Social Sciences version 11.5 (SPSS, Chicago, IL) and Stata 14 (StataCorp, College Station, TX).

Ethical considerations

During the various phases of the outbreak, parents were informed about all measures concerning them and their infants, as were members of the hospital's board of directors. The hospital's ethical committee was contacted, and because data were collected not for research but rather for the purpose of epidemiologic surveillance in the context of an outbreak and managed anonymously, ethical approval was not required.

RESULTS

Evolution of the outbreak

From January 2016 until the first case was identified in June 2016, only 3 cases of infection by *S marcescens* had been detected, and an epidemiologic link among these cases had not been identified. The index case was a 21-day-old male with a conjunctival sample positive for *S marcescens*. After this case, 4 infants presented with *S marcescens* conjunctivitis in July 2016. Between June 2016 and October 2016, cases were found only in the NICU, but in the last week of October 2016, new cases began to appear in the intermediate care area and standard care sections of the Neonatology Unit as well. A total of 59 cases were detected (42 in the NICU and 17 in the remainder of the ward). Thirty-six patients (61.02%) initially presented with colonization, and 23 (38.97%) sustained an infection, 18 (78.27%) with conjunctivitis and 5 (21.73%) with bacteremia. At the end of the outbreak, 26 neonates (44.07%) were just colonized (19 rectal, 4 pharyngeal, 2 bronchial, and 1 umbilical colonization), and 33 (55.93%) had ≥ 1 infections at some point, meaning that 27.78% of the colonized neonates changed from asymptomatic carriers to infected during the outbreak period. The infections detected included 22 cases of conjunctivitis, 9 cases of sepsis, 2 cases of pneumonia, 1 case of bacteremia, and 1 case of encephalitis (> 1 infection site was detected in some patients). Only 1 preterm infant (gestational age, 25 + 3 weeks; birth weight, 922 g), who suffered septicemia and septic shock, died of infection by *S marcescens*, which represents a case fatality of 1.7% for colonized/infected infants and 2.9% when considering only infected infants.

A total of 1,111 neonates were admitted in the Neonatology Unit during the outbreak period (375 in the NICU and 736 in the rest of the ward). Weekly cumulative incidences for the NICU and rest of the ward are shown in Figure 1. The global cumulative incidence during the total period (June 2016 to March 2017) was 11.20 cases per 100 neonates at risk in the NICU and 2.31 cases per 100 neonates at risk in the non-NICU wards. Incidence density was 0.29 case per neonate-month at risk in the NICU and 0.06 case per neonate-month at risk in non-NICU wards. The outbreak peaked in January 2017 in both the NICU and non-NICU wards. In February 2017, abrupt decreases in both cumulative incidence and ID were seen after cessation of admissions in the unit. After reopening the ward, a new increase in the incidence was observed. The outbreak was over by March 2017, when the last case was discharged, and no new cases appeared thereafter.

Microbiology

All *S marcescens* isolates tested for antimicrobial susceptibility were resistant to ampicillin, amoxicillin/clavulanate, cephalothin, cefuroxime, cefoxitin, amikacin, and tobramycin and were susceptible to piperacillin/tazobactam, cefepime, ciprofloxacin, ertapenem, imipenem, meropenem, gentamicin, cotrimoxazole, and tigecycline. The genetic relationships of 25 available strains isolated from clinical (n = 22) and environmental (n = 3) samples identified 2 main lineages highly related to the outbreak (patterns 1 and 2) and 3 other lineages (patterns 3, 4, and 5) not clonally related to the outbreak (Fig 2). Strain 15 was recovered from the water of a sink trap, and strains 22 and 23 were isolated from the water of a sink trap and the drain above the trap.

Interventions

From October 2016 until the end of the outbreak, multiple measures were adopted progressively in an attempt to control the outbreak. These included the creation of a multidisciplinary team, enhancement of and training in contact precautions and hand hygiene (compliance was assessed by trained nurses of the Department of Preventive Medicine by direct methods following the instructions in the World Health Organization's guidelines on hand hygiene in health care and using the Hand Hygiene Observation Tool[9,10]), cohorting for affected infants with dedicated personnel, cleaning and disinfection, and environmental sampling, among others (Table 3).

Environmental investigation

Among the 318 samples obtained (Table 2), only 3 were positive for *S marcescens*, all of them corresponding to siphons from different

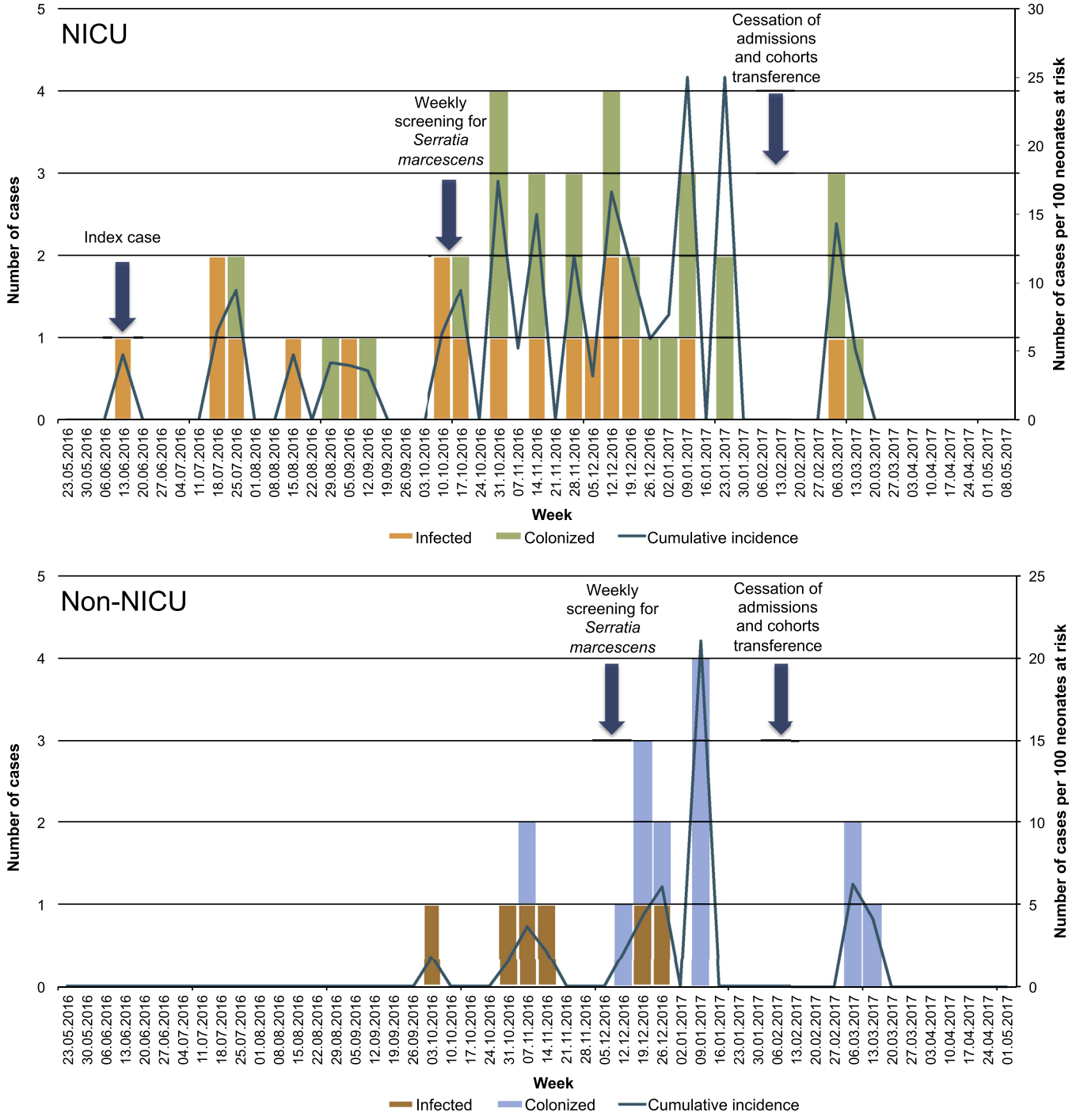

Fig. 1. Number of new cases/week and weekly cumulative incidence in the neonatal intensive care unit (NICU) and non–neonatal intensive care unit (non-NICU) wards.

sections, 1 in the NICU (sample taken on December 21, 2016) and the other 2 in standard care pods (samples obtained on January 20, 2017). The remaining samples were negative for *S marcescens*.

Case-control study

A total of 46 cases (all that had been registered until the analysis was performed) and 63 controls (screened and negative for *S marcescens*) were included in the study (Table 1). Concerning maternal characteristics, the univariate analysis showed significant differences in the use of corticoids before delivery and a history of maternal infection during pregnancy, both of which were higher in the cases. Neonates colonized/infected by *S marcescens* had a significantly lower gestational age and birth weight. Cases were also more frequently affected by heart, hematologic, digestive, and endocrine disorders. Some diagnostic and therapeutic procedures were performed significantly more frequently in cases compared with controls, including radiography; brain, heart, and abdominal ultrasound; invasive and noninvasive mechanical ventilation; transfusions; venous central catheter insertion; parenteral nutrition; and phototherapy.

In the multivariate logistic regression, the factors associated with a significantly increased risk for *S marcescens* infection/colonization

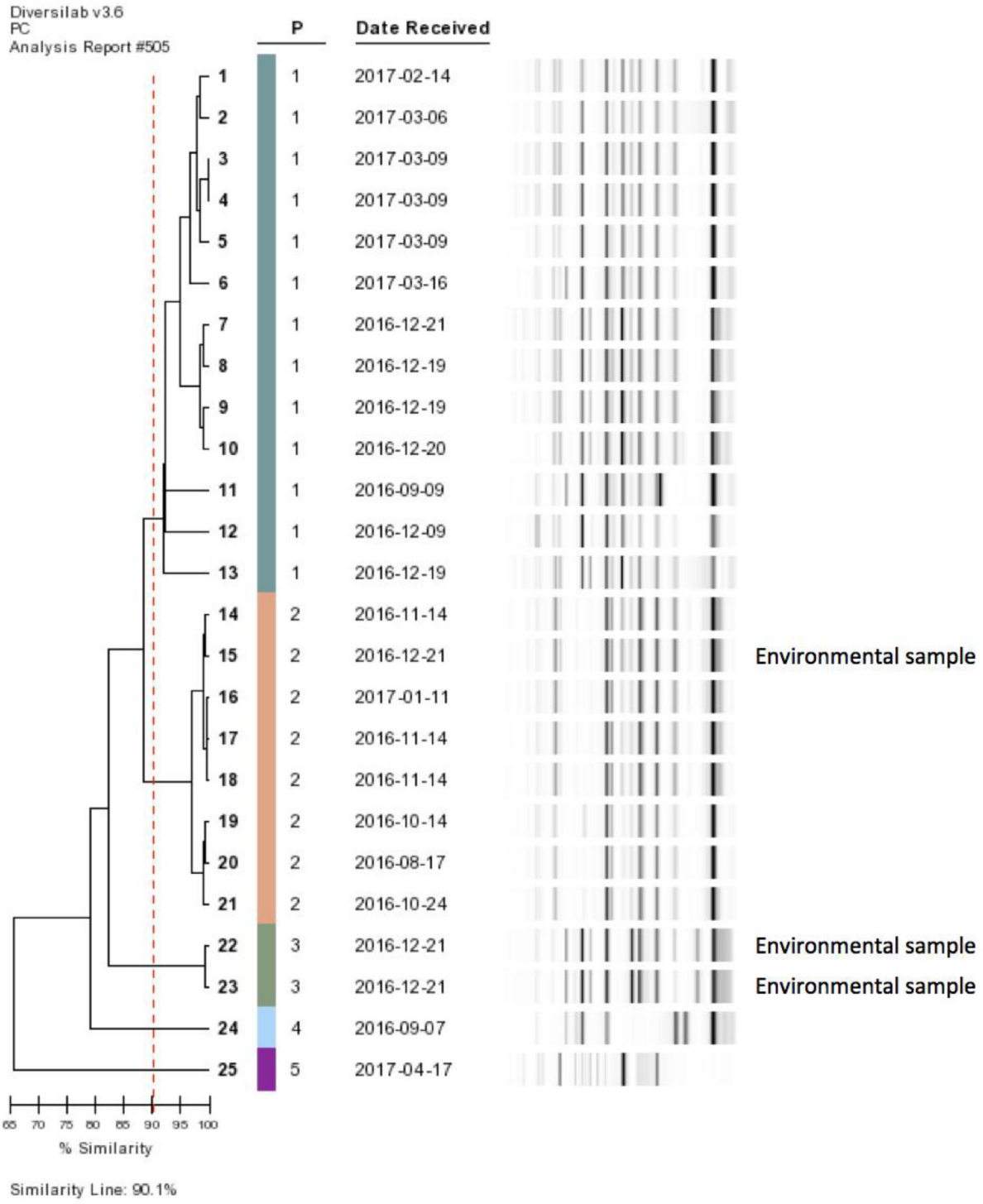

Fig. 2. Dendrogram and virtual gel images representing the repetitive-sequence–based polymerase chain reaction fingerprint patterns of the *Serratia marcescens* isolates.

were receipt of parenteral nutrition (odds ratio [OR], 103.4; 95% confidence interval [CI], 11.9-894.8), radiography (OR, 15.3; 95% CI, 2.4-95.6), and preterm birth (OR, 5.65; 95% CI, 1.5-21.8)

DISCUSSION

We report a large prolonged outbreak of *S marcescens* in the Neonatology Unit of a tertiary hospital in Spain. *S marcescens* is a microorganism that spreads very readily, especially in wards with immunocompromised patients, wards attended by many HCWs, and wards in which numerous interventions are performed, making the Neonatology Unit an ideal setting for an outbreak.

As shown in Figures 1 and 2, the number of cases increased rapidly in both the NICU and non-NICU wards until February 2017, when the multidisciplinary group decided to halt admissions in the Neonatology Unit, pointing to deficiencies in infection control practices. Infants colonized/infected by *S marcescens* acted as a reservoir and the main source; in fact, in February, the transmission risk decreased

Table 3
Sequence of interventions implemented during the outbreak

Interventions	Period
Cohorting for colonized and infected patients by *Serratia marcescens*	July 2016 →
Enhancement of standard precautions and hand hygiene	July 2016 →
Multidisciplinary team creation: neonatologists, preventive medicine specialists, microbiologists, nurses, management team, and contract cleaners	September 2016
Regular meetings	September 2016 →
Environmental sampling	September 2016 →
Incubator cleaning with filtered water and humidifying with sterile water	September 2016 →
Establishment of contact precautions	September 2016 →
Staff training in special measures, including hand hygiene and contact precautions	September, November, and December 2016
Compliance monitoring for hand hygiene and contact precautions (90% adhesion)	October 2016 and January 2017
Prohibition of multidose vials	October 2016
Deep cleaning and disinfection with hydrogen peroxide steam of all sections and common areas	October 2016 and December 2016
Sink drain replacement and particle filter installation	November 2016
Precautions and measures signage at entrances of wards	November 2016
Vigilant nurse at the entrance of the cohorting ward to ensure measures compliance	December 2016 →
Cohorts transference to a different wing	January 2017
Patient surroundings cleaning with chlorinated products twice daily	January 2017 →
Separated areas for affected infants' parents	January 2017 →
Exclusive staff for affected infants	January 2017 →
Daily meetings	January 2017 →
Cessation of admissions	January-February 2017
Pharyngeal screening for health care workers in charge of infants colonized after cleaning and disinfection (all results were negative)	March 2017
Final cleaning and disinfection with hydrogen peroxide steam when the outbreak was considered ended	April 2017

→, onward.

as these infants were progressively discharged or transferred to other NICUs. Cleaning and decontamination with hydrogen peroxide was also performed during this period.

After the unit reopened on March 1, 2017, cases reappeared in the same week in the NICU and non-NICU wards, which may suggest that even though cleaning and decontamination likely eliminated most of the environmental sources, a reservoir could have remained, or *S marcescens* may have been reintroduced into the unit by an HCW and then rapidly spread by hand transmission between other HCWs.

It must be considered that infants assisted in this unit require special care provided by HCWs from other areas of the hospital, which increases the risk of transferring *S marcescens* from other wards (where it is frequently isolated as well) to the Neonatology Unit. In similar outbreaks, the main route of transmission was HCWs' hands,[11-13] which is likely to have occurred in our neonatal unit as well; however, the possibility of hand transmission via parent contact should be taken into consideration.[14]

Despite restricted access to the unit, as well as institution of hygiene measures to avoid cross-transmission, new cases continued to appear. Therefore, the possibility of an alternative reservoir, such as the pharynx in healthy adults, although uncommon, had to be considered as a source of infection instead of hand transmission. For this reason, the multidisciplinary team decided to obtain pharyngeal samples from HCWs who had been in charge of the most recent new cases. Voluntary screening of HCWs was established in agreement with the Department of Occupational Health and the hospital`s leading team. All HCWs agreed to undergo screening. Had pharyngeal samples tested positive, HCWs would have been directed to wear surgical masks while assisting patients; however, all samples were negative for *S marcescens*.

The decision of dedicated personnel to attend the various cohorts was difficult because of both practical issues (eg, not always sufficient staff available) and psychological issues (eg, complaints from staff caring for colonized/infected patients/families). This controversial measure is often difficult to carry out owing to the need for highly trained HCWs in these units and shift work but is very effective in preventing cross-transmission.[15] Consequently, it should be considered after all other measures have failed, having proven effective in other *S marcescens* outbreaks in pediatric patients.[16] In the same way, placing infected patients in a different wing if possible, not only cohorting in different pods, may be recommended as an additional step to facilitate compliance with the previous measures.

Partial or complete closure of the unit also may be necessary to achieve complete control of the outbreak,[17,18] although its feasibility depends on numerous factors, and the effectiveness of this measure has not been proven.[19] Achieving successful control requires a multidisciplinary approach,[20] and because many of the foregoing measures were adopted concomitantly, identifying the most effective ones is difficult. Nonetheless, based on the chronological order, it seem reasonable to suggest that discontinuing admissions, assigning dedicated HCWs, and moving affected patients to a different wing helped achieve complete control of the outbreak.

The fatality rate of this outbreak was very low in comparison with that from other studies.[18,21] This could be explained by an active surveillance that helped identify carriers prior to the development of infection, which made neonatologists especially aware of these patients. However, fatality depends on many factors, such as the complexity of patients assisted, making it difficult to compare among different studies.

Three independent risk factors, both internal and external, have been identified in the multivariate regression model: preterm birth, use of parenteral nutrition, and history of radiography. Prematurity is a previously known risk factor that has been implicated in several outbreaks.[14,18,22] This risk may be related to the incompletely established intestinal microbiota, making preterm infants more susceptible to colonization by microorganisms present in health care facilities.[23] Preterm infants are also more susceptible to infection, owing to an immature immune system and less effective skin and mucosal barriers,[5] which may be weakened as a consequence of invasive procedures that are performed more frequently in preterm infants admitted to NICUs.

Parenteral nutrition has been previously identified as a potential risk factor for acquiring *S marcescens*.[21,24-26] Parenteral nutrition is a nutrient-rich preparation that can provide a favorable growth media for microorganisms and thus requires compounding under highly sterile conditions. Nosocomial bloodstream sepsis infection owing to parenteral nutrition is a potentially fatal complication, with an attributable mortality rate of 11% in neonates.[26] Although no positive

parenteral nutrition samples were found in our study, it should be noted that samples were obtained in March 2017, after the case-control study was performed. Consequently, contaminated parenteral nutrition could have been already administered, or the source of contamination could have been eliminated before samples were collected. However, had parenteral nutrition been the main source of *S marcescens*, we would expect to see many more cases of bacteremia caused by *S marcescens* than were observed during the outbreak. In addition, routine controls were carried out in the hospital pharmacy every month, with no *S marcescens* detection. Thus, we hypothesize that receipt of parenteral nutrition may be a risk factor in terms of increased vulnerability, independent of prematurity.

Finally, with respect to radiography as a risk factor, some studies have found an association between contaminated portable radiology equipment, as used in this ward, and the presence of certain bacteria in intensive care units.[27] In addition, radiography equipment and accessories have been proposed as possible vectors of nosocomial infection.[28] However, in this investigation, no samples from radiologic equipment were positive, which may be explained by the improved cleaning practices during the outbreak and the fact that when the case-control study was conducted in February 2017, the equipment had already been disinfected while the unit was closed.

Strain typing results allow categorizing isolates as identical, highly related but not identical, moderately related, and unrelated. However, these criteria for bacterial strain typing interpretation have been available and validated only for interpreting chromosomal DNA restriction patterns produced by pulse-field gel electrophoresis (PFGE).[29] The main limitation of automated systems for bacteria typing is the lack of validated interpretative criteria, although Ligozzi et al[8] correlated epidemiologic data from an outbreak of *S marcescens* in the NICU with strain typing results produced by PFGE and automated repetitive-sequence–based polymerase chain reaction using the Diversilab system. Some recent studies have applied whole-genome sequencing in investigations of *S marcescens* outbreaks.[30] Although PFGE remains the gold standard for most bacterial species, whole-genome sequencing is a promising and increasingly used tool for strain typing that often provides a higher resolution than other tools.[13]

Contaminated sinks and drainage systems have been identified as stable reservoirs of Enterobacteriaceae, including *S marcescens*.[31] Biofilm-forming bacteria may form reservoirs on the wet surfaces of the pipes, and water splashing from the faucets can create an aerosol effect from the sink's drain, which may contaminate the basin and surrounding surfaces.[32]

Among all environmental samples, only 3 were positive for *S marcescens,* all of which were obtained from siphons located in different pods. Of these, only 1 strain seemed to be related to the outbreak according to the molecular typing results. This may suggest that these findings can be considered as more a consequence than a cause (likely the result of contamination by handwashing or contact with contaminated material in sinks), because cases appeared in other pods as well, and the mechanism through which infants could have been colonized/infected from siphons seem infeasible. Furthermore, cases continued to appear after pieces from sinks and siphons were changed. The strain unrelated to the outbreak could be related to the persistence of a different strain of *S marcescens* inside the biofilm. The formation and establishment of microbial biofilm owing to microbiota in wastewater and patients admitted to the unit over time could lead to the persistence of different species or even diverse bacterial strains of the same species. Therefore, an environmental source was not identified as it was in many other outbreaks caused by this agent.[1]

One limitation of our study is a possible underestimation of carriers during the period when *S marcescens* was screened only in the NICU but not in the other sections. However, for the purpose of the case-control study, to avoid classification bias, we selected controls only from the time that screening for *S marcescens* was being done in the whole unit. Clinical data were obtained from the medical records, and although they are generally exhaustive, it is possible that some variables might not have been correctly registered.

It remains essential to keep these units under close surveillance, given that outbreaks can spread very readily owing to the complexity of cases, the large number of HCWs, and the large number of invasive procedures performed in these units. As a long-term consequence of the outbreak, control measures were enhanced among HCWs as well as patients' relatives, with the number of specialists visiting patients reduced to the minimum required for assistance.

CONCLUSIONS

S marcescens outbreaks in neonatology units tend to be prolonged and difficult to control, and, as has been reported in other outbreaks, we could not identify an environmental source of this outbreak. Owing to the inconclusive results of the environmental investigation as well as the high number of HCWs and procedures performed in the unit, HCWs' hands were the main suspected mechanism of transmission. Measures typically implemented to control nosocomial outbreaks in other units might not be sufficient to control outbreaks in the Neonatology Unit. To achieve rapid control, a multidisciplinary approach is required, intensifying universal precautionary measures and considering stricter measures, such as cohorting, medical attention by dedicated personnel, or closure of the units. Close surveillance of these units remains essential because outbreaks spread very easily owing to the high number of HCWs attending these particularly vulnerable patients and the invasive procedures performed during their care.

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