Enferm Infecc Microbiol Clin. 2016;34(10):645–651

Enfermedades Infecciosas y Microbiología Clínica

www.elsevier.es/eimc

Original article

A six-month *Serratia marcescens* outbreak in a Neonatal Intensive Care Unit

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ARTICLE INFO

Article history:
Received 26 October 2015
Accepted 11 January 2016
Available online 18 February 2016

Keywords:
Intensive Care Units
Neonatal
Nosocomial Infection
Healthcare-associated infection
Disease outbreak
Serratia marcescens

ABSTRACT

Objective: To investigate a *Serratia marcescens* (*S. marcescens*) outbreak in a Neonatal Unit in a tertiary university hospital.
Methods: Descriptive study of children admitted to the Unit with *S. marcescens* infection from November 2012 to March 2013. Conventional microbiological methods for clinical and environmental samples were used. The clonal relationship between all available isolates was established by molecular methods. A multidisciplinary team was formed, and preventive measures were taken.
Results: S. marcescens was isolated from 18 children. The overall attack rate was 12%, and the case fatality rate in the Intensive Care Unit was 23.5%. The most prevalent types of infections were pneumonia (6), conjunctivitis (6), and bloodstream infection (5). Clinical isolates and environmental isolates obtained from an incubator belonged to a unique clone. The clonal relationship between all *S. marcescens* strains helped us to identify the possible source of the outbreak.
Conclusion: Isolation of *S. marcescens* from stored water in a container, and from the surface of an incubator after cleaning, suggests a possible environmental source as the outbreak origin, which has been perpetuated due to a failure of cleaning methods in the Unit. The strict hygiene and cleaning measures were the main factors that contributed to the end of the outbreak.

Brote epidémico por *Serratia marcescens* en una Unidad de Cuidados Intensivos Neonatales

Palabras clave:
Unidades de Cuidados Intensivos
Neonatales
Infección nosocomial
Infección asociada a la asistencia sanitaria
Brote epidémico
Serratia marcescens

RESUMEN

Objetivo: Investigar un brote por *Serratia marcescens* (*S. marcescens*) en una unidad de neonatología en un hospital universitario de tercer nivel.
Método: Estudio descriptivo de los pacientes ingresados en la Unidad de noviembre de 2012 a marzo de 2013. Se usaron métodos microbiológicos convencionales de muestras clínicas y ambientales. La relación clonal de los aislados disponibles se llevó a cabo mediante estudio molecular. Se formó un equipo multidisciplinar a partir del cual se tomaron las medidas preventivas.
Resultados: Se aisló *S. marcescens* en 18 niños. La tasa global de ataque fue del 12% y la letalidad en la Unidad de Cuidados Intensivos llegó al 23,5%. Los tipos de infección más frecuentes fueron la neumonía (6), conjuntivitis (6) y bacteriemia (5). Tanto las muestras clínicas como las ambientales obtenidas de una incubadora pertenecían a un único clon. La relación clonal entre todas las cepas de *S. marcescens* permitió identificar la posible fuente de infección del brote.

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http://dx.doi.org/10.1016/j.eimc.2016.01.006

Conclusión: El aislamiento de *S. marcescens* en agua almacenada en un contenedor y en la superficie de una incubadora tras su limpieza sugiere una posible fuente ambiental como el origen del brote, perpetuado por fallos en los métodos de limpieza en la Unidad. El cumplimiento estricto de la higiene de manos y mejora en la limpieza fueron los principales factores que contribuyeron a la finalización del brote.

Introduction

Serratia marcescens is considered an opportunistic pathogen able to produce serious infections in Neonatal Intensive Care Units (NICU) with high morbidity and mortality, and the latter could reach 44% in low birthweight.[1]

S. marcescens can survive in damp environments and colonize the respiratory and/or gastrointestinal tract, as well as in hands of patients and healthcare workers, accounting for 5–15% of all nosocomial infections in NICU, and has been isolated for a long time in some nosocomial outbreaks.[2,3]

Strict preventive measures are required to control these infections, primarily because of the great difficulty to eradicate this bacterium from these Units during an outbreak. Secondly, some of these strains have the ability to be resistant to multiple antibiotics which may hinder their treatment, and this fact could have severe consequences in low birthweight infected patients.[4] In this paper, we describe the epidemiological investigation of a *S. marcescens* nosocomial outbreak at the NICU that allowed us to identify a possible environmental source and undertake the preventive measures to control it, as well as to define areas for improvement in order to prevent the appearance of new cases.

Methods

Area and period of study

Since November 2012 to March 2013, a *S. marcescens* nosocomial outbreak occurred at the Neonatal Unit (NU) of the University Hospital Virgen del Rocío in Seville, which it is also a regional tertiary reference center for other provinces of Andalusia (Spain). The NU is newly built since 2011, and is subdivided into three areas: General Neonatology (32 beds), Intermediate Care Unit (ICU) with 16 beds, and Neonatal Intensive Care Unit (NICU) with 12 beds.

This Unit served a total of 1790 inpatients in 2011, with an average stay of 8.7 days per patient. None of the three Units share health personnel except the pediatric postgraduate residency training on duty.

Epidemiological research

The 11th of December 2012, the outbreak was declared when five cases were already diagnosed in the NICU. Subsequently, more cases were identified at the NICU and ICU, with a total of 18 children involved, and the last case being detected in March 2013.

Study population included all patients infected and admitted to NICU and ICU from the first detected case. Case definition was any patient admitted to NICU or ICU, since November 2012 to March 2013, with a *S. marcescens* positive culture.

According to the site of infection and the clinical symptoms, we classify children as confirmed cases (when *S. marcescens* was isolated from sterile fluid samples and/or from non-sterile sites and clinical symptoms were present) and colonized/carrier cases (when the bacteria was isolated from non-sterile sites in the absence of signs and/or symptoms of infection).

The Service of Preventive Medicine collected cases data by individual inquiry protocol. Variables including personal identifiable information, intrinsic risk factors and those associated with infection and related procedures were collected.

Environmental study

During 2013, an extensive environmental study, which included 42 samples, was conducted to search for a potential source of *S. marcescens*.

Microbiological study

Environmental and clinical samples were processed following the Proceedings of the Spanish Society of Clinical Microbiology and Infectious Diseases, SEIMC (Environmental Microbiological 2nd Edition (42), 2012) and incubated for 7 days. Environmental samples were collected at three different dates: November 2012, February 2013 and December 2013.

The *S. marcescens* isolates were preliminarily identified by mass spectrometry (MALDI-TOF-MS Brucker®, Microflex LT instrument software and database Flexcontrol Biotyper 3.0 2.0 (Bruker Daltonics)). Isolates were stored at −70° until the realization of molecular study.

The fenotypical identification and susceptibility testing was performed with MicroScan Walkaway system (Beckman Coulter Inc.). Susceptibility testing was performed by standard methods in accordance with the European Committee on Antimicrobial Susceptibility Testing – (EUCAST) performance standards.

On January 29, 2013 a study of conjunctival and rectal carriage of *S. marcescens* to the 14 inpatient children of the Unit was performed. Pulsed field electrophoresis (PFEG) analysis on all the available strains isolated from clinical and environmental samples was performed, following the modified protocol described by Shi Zhi-yuan et al., using the *Spe*I restriction enzyme.[5] Electrophoresis patterns obtained were captured with GelDoc™ XR + with Image Lab™ Software. These patterns were compared using the Bioinformatics FPQuest™ Software system, BIO-RAD. Dendogram was performed by the Dice coefficient and UPGMA (tolerance 2%), and the pulsotypes obtained were interpreted according to the Tenover criteria.[6]

Statistical analysis

The attack rate (AR) or *S. marcescens* infection incidence of inpatient children at the Unit (number of new cases among all patients at risk), the case fatality rate (number of deaths among infected) and *S. marcescens* mortality (deaths of all patients at risk) were calculated. The risk population was considered in all the inpatient children at the affected Units during the outbreak period.

A time, place and person descriptive analysis of the cases was performed. Categorical variables were represented by frequency tables and quantitative numerical summaries [mean and standard deviation, median and interquartile range (IQR)].

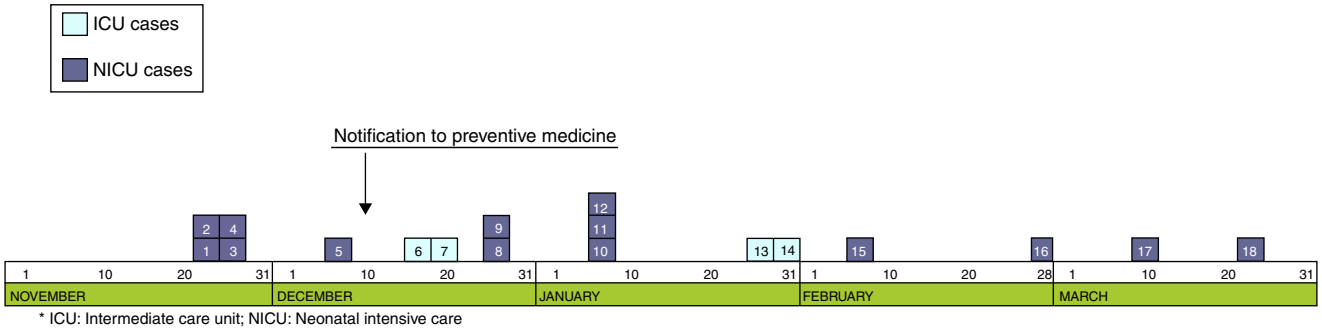

Fig. 1. Epidemic curve. November 2012–March 2013.

Results

Outbreak description

On the 23rd of November, the outbreak started at the NICU, and since then, clusters of new cases were detected until March 25 of 2013, when the last case was diagnosed. In the first period, from November 23rd to December 7th, a cluster of five cases with a high AR (41.6%) and mortality (40%) was identified in NICU (Fig. 1). Lately, new cases appeared intermittently with longer period among them, and the last case was detected on March 25, 2013. The global AR throughout the epidemic period has been 12%, ranging from 9.3% in ICU to 13.5% in NICU.

Although two Units have been affected, the outbreak began at the NICU where most of the new cases were detected throughout all the epidemic period including 13 (72.2%) of the 18 cases. Ten cases (55.6%) were located at the area where the incubators 57–63 were set (Fig. 2). In the second half of December, five cases were diagnosed at the ICU after the transfer of two cases from NICU to ICU.

S. marcescens was isolated from 16 infected and 2 colonized patients, 13 male (72.2%) and 5 female children (27.8%), with a median age of 24 days (IQR 12–45). Eighty-seven percent of patients were at the Unit since its birth, and the patient's median stay in the Unit when the infection succeeds was of 19 days (IQR 11–46). Prematurity (56%) was the first cause for admission followed by congenital malformation (22.3%). Sixty-seven percent received antibiotic therapy (median 14 days; IQR 7–25.5), and the most commonly used antibiotics were ampicillin, gentamicin, vancomycin and meropenem.

The most prevalent clinical disease patterns were distributed as follows: six pneumonia, six conjunctivitis, five bloodstream infection and one urinary tract infection. In six cases, there was coinfection with other enterobacteria (Table 1). Additionally, in six cases, respiratory tract colonization was present.

Clinical evolution was favorable in 15 cases but three of the bloodstream infection cases died. The overall case fatality rate for the entire epidemic period was 16.6% (23% in NICU) and the death rate of infection in *S. marcescens* exposed population was 2% (3% in NICU).

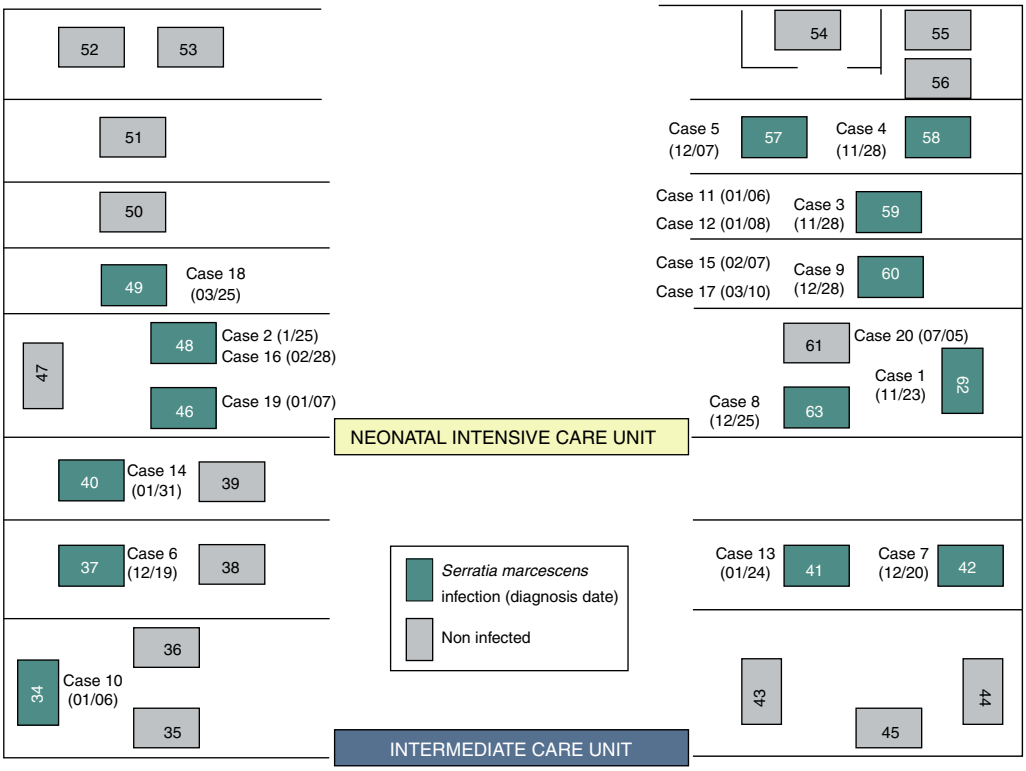

Fig. 2. Geographical distribution of cases at the Neonatal Intensive and Intermediate Care Units.

Table 1
Description of patients with *Serratia marcescens* infection.

	Age (days)[a] / gender	Birthweight (g)	Gestational age (sem)	Cause of admission	Length of stay[b]	Date of infection	Isolated bacteria	Type of infection	Antibiotic therapy[c]	Clinical course
CASE 1	9/M	1023	29	Preterm	9	11.23.12 11.28.12	SM	Bloodstream infection Pneumonia	7	Recovery
CASE 2	57/F	680	25	Preterm	57	11.25.12 12.08.12	SM	Bloodstream infection	24	Deceased
CASE 3	39/M	760	25	Preterm	22	11.28.12	SM	Urinary tract infection	21	Recovery
CASE 4	70/F	2423	28	Preterm laser therapy	7	11.28.12	SM, PS	Colonization of the respiratory tract	0	Recovery
CASE 5	6/M	1400	29	Preterm	5	12.07.12	SM, EC	Bloodstream infection	5	Deceased
CASE 6	12/M	3670	39	Congenital cardial malformation	12	12.18.12	SM, PS	Pneumonia	0	Recovery
CASE 7	14/M	1480	30	Preterm	14	12.20.12	SM	Conjunctivitis	12	Recovery
CASE 8	45/F	935	28	Preterm	45	12.25.12	SM, EC	Pneumonia	26	Recovery
CASE 9	52/F	995	27	Preterm	48	12.28.12	SM, PS	Bloodstream infection	32	Recovery
CASE 10	31/M	1352	30	Preterm, sepsis	31	01.06.13	SM	Conjunctivitis	11	Recovery
CASE 11	10/F	1326	32	RDS, IGR	10	01.06.13	SM	Conjunctivitis	0	Recovery
CASE 12	27/M	3040	37	RDS, hypoglycemia	25	01.10.13	SM	Pneumonia	16	Recovery
CASE 13	15/M	1583	29	Preterm	15	01.24.13	SM	Conjunctivitis	3	Recovery
CASE 14	15/M	3167	37	Esophageal atresia	14	01.31.13	SM	Conjunctivitis	0	Recovery
CASE 15	9/M	3385	37	Esophageal atresia	6	02.07.13	SM	Pneumonia	0	Recovery
CASE 16	28/M	1324	31	Preterm Histiocytosis	28	02.28.13 03.27.13	SM	Bloodstream infection Conjunctivitis	27	Deceased
CASE 17	46/M	1800	33	Preterm neumopathy	46	03.10.13 03.30.13	SM	Colonization of the respiratory tract	3	Recovery
CASE 18	21/M	3470	40	Congenital cardial malformation	12	03.25.13	SM, KP, PS	Pneumonia	0	Recovery

[a] Age at infection.
[b] Length of stay before infection.
[c] Number of days of antibiotic therapy before infection. M: male; F: female; RDS: respiratory distress syndrome; IGR: intrauterine growth retardation. SM: *Serratia marcescens*; KP: *Klebsiella pneumoniae*; PS: *Pseudomonas aeruginosa*; EC: *Enterobacter cloacae*.

Table 2
Preventive measures undertaken to control the outbreak.

Recommendations and main results
Measures related to storage and transport of water used for incubators
◦ Elimination of the water transportation circuit used to fill incubators and replaced by sterile water
◦ Revision, sampling and disinfection of the water tank
SM carriers study
Conjunctival and rectal swabs were taken from all patients admitted to ICU and NICU for more than 24 h. A total of 28 samples (14 conjunctival and 14 rectal) were studied from January 28th to February 2nd, 2013
Hygiene and isolation measures
◦ Contact isolation of cases and carriers
◦ Restriction of visits and exclusive healthcare personnel for cases
◦ Hand hygiene direct observation. 156 workers were studied (48.7% nurses, 25.6% doctors, 25.6% nursing auxiliaries) and a total of 207 hand hygiene opportunities were observed. The overall compliance with hand hygiene was 82.1% (optional: 93.5%; nurses: 79.5%; nursing auxiliaries: 81.8%)
Hygiene measures in ophthalmic examination an blefarostates disinfection
Revision of equipment and disinfection techniques used in the ophthalmic examination and hygiene measures taken by the medical staff. After literature review, it was decided to sterilize blefarostates instead of their disinfection
Empiric antibiotic therapy
Review and update of empiric antibiotic therapy protocols
Cleaning measures
All antiseptics used in the Unit during the outbreak were replaced by new ones. Some of them were sampled for microbiological study
Cleaning protocols and types of disinfectants used in the Unit were reviewed and updated
Development of a checklist to evaluate the cleaning measures

SM: *Serratia marcescens*; ICU: Intermediate Care Unit; NICU: Neonatal Intensive Care Unit.

Preventive measures

The first preventive measures adopted to prevent cross-transmission of infection were prospective surveillance of patients admitted to NICU, extreme cleaning measures and emphasized the strict compliance hand washing on healthcare workers.[7]

Other preventive measures such as conducting environmental microbiological study, review and logging cleaning steps, contact isolation, restriction of visits, study of *S. marcescens* carriers, review of empirical antibiotic policy at the NICU and direct observation of hand hygiene of healthcare workers were subsequently taken (Table 2).

Twenty-three *S. marcescens* strains were isolated, 18 from clinical samples and 5 from environmental samples. Among the later, two of them were from the incubator surfaces, one from the water container and likewise, two *S. marcescens* strains from the pipes of two different sinks.

None of the 28 cultured samples to detect *S. marcescens* carriage from inpatient children were positive.

Microbiology and molecular study

All the *S. marcescens* isolates were resistant to ampicillin, amoxicillin/clavulanate, cephalothin, cefazolin, amikacin and tobramycin and susceptible to ciprofloxacin, cefepime, imipenem, ertapenem, gentamicin, piperacillin/tazobactam, cotrimoxazole and tigecycline. The dendrogram created by mass-spectrometry analysis software grouped the first seven strains of the outbreak into one cluster, and allowed us to rule out any clonal relationship with previous isolates (Fig. 3).

The molecular study identified two main lineages of *S. marcescens* (Clon A and B). The Clon A included all the 2012 strains isolated before the outbreak's declaration and the strain isolated from the water container (this strain was not identical to those from the clinical strains though highly related – 97%, suggesting a common lineage). The Clon B included all the strains involved

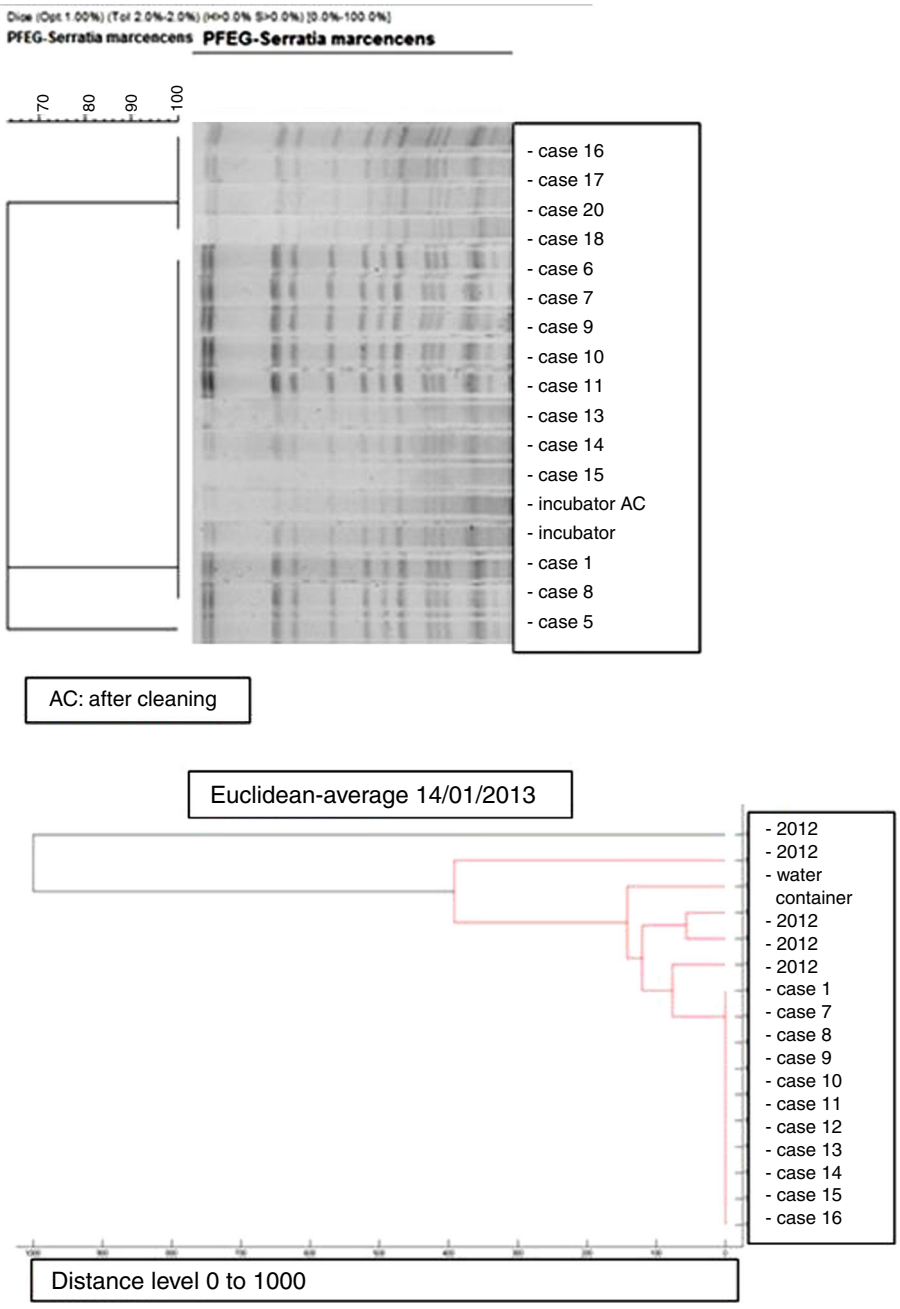

Fig. 3. PFGE analysis of 17 *Serratia marcescens* strains including outbreak cases and incubator strains (before and after cleaning) and the dendrogram of the proteins profiles created by mass-spectrometry analysis of 18 strains of *Serratia marcescens* (including 11 outbreak cases – one strain per patient, two environmental samples and five clinical isolates of the year 2012. Correlation distance measure with the average linkage Algorithm. Maldi-TOFF. Bruker™.

in the outbreak cases and those isolated from the incubator, before and after cleaning (Fig. 4).

Discussion

In recent years, *S. marcescens* has been frequently involved in outbreaks at NICU that are characterized for its quick spread, causing severe complications, and with a highly variable incidence and duration.[3,8,9]

Our work, in contrast with other studies, emphasized the high number of confirmed cases in a short period of time (4 months) and the high lethality of the infection in the first epidemic wave (close to 40%), especially in bloodstream infection cases, exceeding what is reported in other outbreaks.[4,10,11]

Risk factors that contribute to severe forms of *S. marcescens* infections in neonates are: low gestational age and birthweight, longer hospital stays, prior antibiotic therapy and use of invasive devices such as central or percutaneous venous catheter, orotracheal intubation or parenteral nutrition.[2,8,9,12–14] In our study, more than half of the infected patients shared the aforementioned factors such as birthweight less than 1500 g, gestational age less than 35 weeks and prior prolonged antibiotic therapy and length of stay.

An environmental source is not detected in most of the studies, and it is assumed that patients act as a reservoir of infection, and through cross-transmission the bacteria spreads to other patients.[3,8,14] However, in our study, although we cannot unequivocally identify the source, there are some facts that suggest their existence. Firstly, because of the clonal relationship between the water container *S. marcescens*, strain with those isolated from the

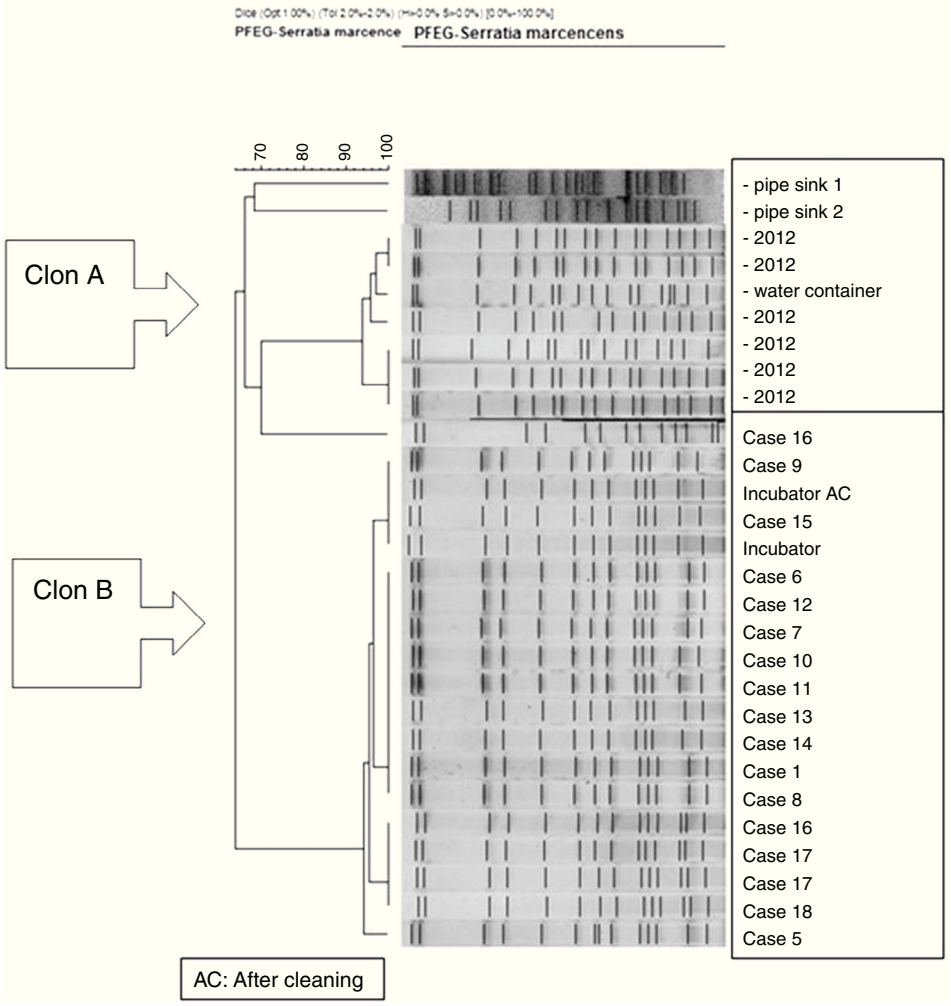

Fig. 4. PFGE analysis of 28 *Serratia marcescens* strains including clinical and environmental samples (Dice coefficient, tolerance 2%).

blood cultures in months prior to the outbreak. Secondly, no rectal, neither conjunctival *S. marcescens* carriers, were detected during the outbreak. Finally, the epidemic curve distribution with a high number of cases in the first outbreak wave suggest that a common environmental source initiated it, and the infected patients acted later as reservoir of the bacteria. The *S. marcescens* ability to survive in the gastrointestinal and/or respiratory tract of infected patients for long periods of time also supports this hypothesis, as can be demonstrated in the 16th and 17th patients, where the same strain was isolated twice, one month apart (Table 2), and by the skin persistence of *S. marcescens* in the hands of healthcare workers that could have contributed to maintain its spread.[1,8,15,16]

Additionally, the effect of overcrowding and understaffing may have contributed to the persistence of the outbreak in time as previously described.[8] In the first epidemic period, where the largest number of cases and higher lethality occurred, there was a temporal absence of four resident physicians in the Intensive and Intermediate Care Units.

Rapid implementation of recommended preventive measures, designed by the multidisciplinary team, is essential in controlling the outbreak and its progression. The contact isolation of cases, exclusiveness of healthcare workers attending the cases, reinforcement on hand hygiene and cleaning are the most referred measures by other authors.[3,9]

Moreover, the availability of PFEG and mass spectrometry played an important role in the outbreak investigation. It allowed us to demonstrate that all cases belonged to a single strain which was the same as that isolated from the water container. This contrasts with other studies where different strains are involved.[4,10,12,13]

Although it is usually assumed that ICU are adequately cleaned, it is known that only about 40% of the areas close to the patient are being disinfected according to the protocols.[17] As it happened in our study, where two identical *S. marcescens* strains were isolated before and after the incubator cleaning, it highlighted a failure in disinfection methods and prompted us to review and update the cleaning protocols and develop an evaluating method to ensure its compliance.

Carling et al. claimed that the thoroughness of disinfection can be improved by up to 82%, which may result in a decrease of 40% in the transmission of multiresistant microorganisms.[17] Furthermore, several guidelines suggest that hospitals should monitor the cleaning tasks, especially on surfaces close to the patient and healthcare workers, and it should be performed by trained personnel.[18–21]

The high number of cases of conjunctivitis also led us to review the disinfection methods of the blefarostates used in the newborn ophthalmologic examinations and decided to sterilize them instead of their disinfection (they usually where disinfected and reused in different patients in the same day).

Other control measures applied in *S. marcescens* outbreaks reported in the literature have been the closure of the Unit and the study of *S. marcescens* carriers among healthcare workers.[13,14,22] However, the closure of the Unit not always guarantees the absence

of new cases after reopening, and there is only one study published in 2006 that detected the same strain of *S. marcescens* which caused the outbreak in the hands of a healthcare worker.[16] In our study, hand hygiene (HH) compliance of healthcare workers was higher than what has been published in a recent systematic review, whose median value is at a 40%,[23] compared to the 80% obtained in our study. However, we should take in to account that the observation method was made during the outbreak investigation, a situation that could motivate workers to be stricter with the HH. This fact, combined with the absence of *S. marcescens* carriers, led to the decision of not studying the bacterial contamination of the hands of the Unit staff.

In conclusion, we emphasize the importance of molecular study and training of the multidisciplinary team who were determining factors in the control and management of the outbreak, both for the development of the study hypothesis and mechanism of transmission of infection, and the implementation of the appropriate preventive measures.

Based on lessons learned, we highlight the importance of monitoring the cleaning measures in ICU, since we tend to minimize their importance in the transmission of nosocomial infection.

Funding

No financial support was given for this study.

Conflict of interest

All authors report no conflicts of interest relevant to this article.

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