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Outbreak of a Cluster with Epidemic Behavior Due to *Serratia marcescens* after Colistin Administration in a Hospital Setting

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***Serratia marcescens* causes health care-associated infections with important morbidity and mortality. Particularly, outbreaks produced by multidrug-resistant isolates of this species, which is already naturally resistant to several antibiotics, including colistin, are usually described with high rates of fatal outcomes throughout the world. Thus, it is important to survey factors associated with increasing frequency and/or emergence of multidrug-resistant *S. marcescens* nosocomial infections. We report the investigation and control of an outbreak with 40% mortality due to multidrug-resistant *S. marcescens* infections that happened from November 2007 to April 2008 after treatment with colistin for *Acinetobacter baumannii* meningitis was started at hospital H1 in 2005. Since that year, the epidemiological pattern of frequently recovered species has changed, with an increase of *S. marcescens* and *Proteus mirabilis* infections in 2006 in concordance with a significant decrease of the numbers of *P. aeruginosa* and *A. baumannii* isolates. A single pulsed-field gel electrophoresis (PFGE) cluster of *S. marcescens* isolates was identified during the outbreak. When this cluster was compared with *S. marcescens* strains ($n = 21$) from 10 other hospitals (1997 to 2010), it was also identified in both sporadic and outbreak isolates circulating in 4 hospitals in Argentina. In132::IS*CR1*::$bla_{CTX\text{-}M\text{-}2}$ was associated with the multidrug-resistant cluster with epidemic behavior when isolated from outbreaks. Standard infection control interventions interrupted transmission of this cluster even when treatment with colistin continued in several wards of hospital H1 until now. Optimizing use of colistin should be achieved simultaneously with improved infection control to prevent the emergence of species naturally resistant to colistin, such as *S. marcescens* and *P. mirabilis*.**

Serratia marcescens is a Gram-negative, facultative anaerobic bacillus of the *Enterobacteriaceae* family that survives in environments and reservoirs such as drinking water, pipes, and hospital disinfectants as well as in medical instrumentation, among other locations (1). This organism is able to colonize the human gastrointestinal tract and skin for extended periods, being transmitted predominantly by person-to-person contact. Pneumonia, bloodstream infections, meningitis, and ocular and urinary tract infections can result from an *S. marcescens* infection (2–6). *S. marcescens* is also well known as a nosocomial pathogen and has been responsible for outbreaks, particularly in critically ill neonates and patients in intensive care units (2, 7–9). Multidrug-resistant *S. marcescens* strains are reported to cause more-invasive infections and tend to spread rapidly in nosocomial environments (10–13). Recently, several fatal cases associated with nosocomial infections of this pathogen have been reported around the world (14, 15). Among other antibiotics, it is naturally resistant to tetracycline, amoxicillin, amoxicillin-clavulanate, cephalothin, and colistin (16). The latter antibiotic is usually administered as a last-resort antibiotic for treatment of multidrug-resistant *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* infections in Argentina and many other countries (17).

We report the investigation and control of the increasing frequency, including an outbreak, of multidrug-resistant *S. marcescens* infections at hospital H1 in Argentina since the inception of colistin use in 2005. We have determined the genetic relationship of *S. marcescens* isolates of the outbreak, and we compared the pulsed-field gel electrophoresis (PFGE) profiles with those of epidemiologically well-characterized isolates from outbreaks and sporadic sources from other 10 hospitals in Argentina (1997 to 2010). The frequencies of other relevant species such as *P. aeruginosa*, *A. baumannii*, *K. pneumoniae*, and *Proteus mirabilis* collected from hospital H1 during the period 2002 to 2011 were also statically analyzed. The usage of colistin was revised from the beginning of its administration at hospital H1. In addition, we evaluated the presence of complex class 1 integrons and β-lactamases associated with horizontal genetic transfer of *S. marcescens* isolates.

MATERIALS AND METHODS

Characterization of the institution and epidemiological features of the outbreak. The study was conducted in a hospital of high complexity with 308 beds, located in the city of Lanús, Province of Buenos Aires, Argentina (hospital H1). A retrospective analysis of the database of the Microbiology Laboratory indicated a progressive increase of *S. marcescens* infections since 2006 in several wards of hospital H1, with an outbreak by this organism from November 2007 to April 2008. Consequently, the outbreak has been studied, including case identification and review of medical records, environmental cultures, patient surveillance cultures, and personnel hand cultures. A total of 50 samples of *S. marcescens* from 44 patients

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were recovered from November 2007 to April 2008. Thirty-two isolates were considered to represent infections of this organism, 6 isolates represented colonization, and the remaining 12 isolates had inadequate data to determine the infection or colonization source. Among the samples studied, 54% were from blood and central catheters associated with the use of vascular access. The remaining isolates were from tracheal aspirates ($n = 2$), bronchoalveolar fluid ($n = 5$), sputum ($n = 2$), pleural liquid ($n = 3$), urine ($n = 9$), and skin and soft tissue ($n = 2$).

Statistical analysis. Data corresponding to the incidence (by year) of isolation of *S. marcescens* and of the most frequent Gram-negative bacilli collected in hospital H1 (*P. aeruginosa*, *A. baumannii*, and *K. pneumoniae*) as well as of *P. mirabilis*, which is also a naturally resistant colistin species, were statistically evaluated using a two-way analysis of variance and *post hoc* least significant difference (LSD) mean comparison analyses.

Bacterial isolates selected for molecular studies. Nine representative isolates of *S. marcescens* from the outbreak collected between November 2007 and April 2008 from hospital H1 were selected for molecular studies. They were compared to other isolates belonging to well-characterized outbreaks at different hospitals (H2 and H3). Also, 20 sporadic isolates (from hospitals H1, H2, and H4 to H11) were included in this study (Table 1). Hospitals H1 to H11 were from 3 provinces of Argentina separated by more than 250 km (Buenos Aires, Santa Fe, and Entre Ríos). Isolates were identified at the species level using standard biochemical tests and by amplified ribosomal DNA restriction analysis for identification of *Serratia* genomic species (24). Until used, isolates were frozen at −80°C in brain heart infusion (BHI) (Difco Laboratories, Detroit, MI) supplemented with 20% (vol/vol) glycerol.

Antimicrobial susceptibility tests. The disk diffusion method was performed in agar as recommended by the CLSI (25). Screening methods for the detection of extended-spectrum β-lactamases (ESBLs) and carbapenemases were performed both following the guidelines established in CLSI standards and according to recommendations of the Antimicrobial Subcommittee of the Sociedad Argentina de Bacteriología, Micología y Parasitología Clínica (25, 26). The antimicrobial agents included were ampicillin, cephalothin, amoxicillin-clavulanic acid, cefotaxime, ceftazidime, gentamicin, amikacin, piperacillin-tazobactam, cefepime, nalidixic acid, ciprofloxacin, trimethoprim and sulfamethoxazole, imipenem, and meropenem (Oxoid Laboratories, Dartford and Perth, United Kingdom).

PFGE. Genomic DNA embedded in agarose plugs was obtained as previously described in the PulseNet Standardized Laboratory Protocol for *Escherichia coli* O157:H7, *Salmonella* serotypes, and *Shigella sonnei* (27). DNA was digested with XbaI (Fermentas, Lithuania), and digests were resolved by pulsed-field gel electrophoresis (PFGE) (CHEF-DR III system; Bio-Rad, Richmond, CA) using electrophoresis conditions of an initial switch time (IST) of 5 s, a final switch time (FST) of 35 s, and a running time (RT) of 17 to 18 h at 6 V/cm and 14°C. *Salmonella* Braenderup H9812 was run in multiple positions in each gel (4 lanes in a 15-well gel) to be used as the standard for the PFGE gels in accordance with PulseNet International protocols (18). To resolve the PFGE profiles of some isolates that were recorded as nontypeable, these strains were tested again with the addition of 50 μM thiourea to the electrophoresis buffer, as recommended by other authors (19, 20, 28). PFGE profiles were subjected to computer-assisted DNA fingerprint analysis using BioNumerics version 4.6 software (Applied Maths, Sint-Martens-Latem, Belgium), and dendrograms were constructed using UPGMA (unweighted-pair group method using average linkages), the Dice coefficient, and a 1.5% band position tolerance window. Isolates showing 85% similarities were considered related.

Identification of genes associated with antibiotic resistance. Genomic DNA was extracted with a Wizard genomic DNA purification kit (Promega, Madison, WI). PCR amplifications using total DNA were performed according to the instructions of the manufacturer (Promega, Madison, WI) using specific primers for evaluating the presence of antimicrobial resistance determinants associated with horizontal genetic transfer and commonly found in Gram-negative bacterial multidrug-resistant clinical isolates from Argentina: (i) integron integrase genes (*intI1*) (21), (ii) relevant β-lactamase genes ($bla_{\text{CTX-M-2}}$, $bla_{\text{SHV-like}}$, $bla_{\text{PER-2}}$, $bla_{\text{GES-like}}$, $bla_{\text{VEB-like}}$, $bla_{\text{VIM-like}}$, $bla_{\text{IMP-like}}$, and $bla_{\text{SPM-like}}$) (22), (iii) *qnrB* genes (23), and (iv) complex class 1 integrons (21). DNA products were analyzed by conventional agarose gel electrophoresis and confirmed by sequencing.

RESULTS

Description of the outbreak in hospital H1. A retrospective study indicated a significant increase of *S. marcescens* infections from 2005 ($n = 13$) to 2007 ($n = 41$) ($P < 0.0001$) (Fig. 1 and Table 2). Based on the concept of "outbreak," which is an increase in nosocomial infection rates above that noted in the past, we could identify an outbreak in the clinical sector from November 2007 to April 2008 ($n = 50$ isolates). The baseline rate of *S. marcescens* infection or colonization in 2005 was 1.0 cases/month, in 2006 the rate increased to 4 cases/month, and during the outbreak in 2007 to 2008 the rate reached 10 cases/month. During the outbreak there were 50 cases of infection or colonization caused by *S. marcescens*, with 32 identified as infections; 97% of the 50 cases of infection or colonization were assisted in the clinical area, suggesting horizontal dissemination from a common source of infection. The mean age of the patients was 53 years (ranging from 19 to 87 years). Seventeen episodes of infection were found in blood culture samples possibly associated with previous vascular access. Fourteen patients died during the outbreak; 13 deaths were attributed to multidrug-resistant *S. marcescens* infection (40% of infected patients). It should be noted that no data were available about the outcome for 22% (7/32) of the infected patients, so it was not possible to know their evolution, suggesting that the attributable mortality could be greater than 40%.

All the *S. marcescens* isolates from the outbreak showed the same multidrug resistance profile, corresponding to resistance to ampicillin, cephalothin, amoxicillin-clavulanic acid, ceftazidime, cefotaxime, cefepime, piperacillin-tazobactam, amikacin, gentamicin, nalidixic acid, ciprofloxacin, trimethoprim, and sulfamethoxazole and susceptibility only to imipenem and meropenem (Table 1). *S. marcescens* isolates recovered before and after the outbreak were susceptible to most of the antibiotics assayed (Table 1).

Administration of colistin in hospital H1. The use of colistin began in 2005 with purchase following a request for the treatment of severe adult postsurgical meningitis due to carbapenem-resistant isolates of *A. baumannii.* Since 2007 it has been incorporated in the pharmacotherapy of the hospital as one of the antibiotics of restricted use for treatment of carbapenem-resistant *A. baumannii*, *P. aeruginosa*, or *K. pneumoniae* infections. The administration of colistin from 2007 to 2011 has been documented, and it ranged from 0.5 to 1.5 defined daily doses (DDD).

Analysis of the incidence of infections from 2002 to 2011 in hospital H1. A two-way analysis of variance with categories of bacterial species (*S. marcescens*, *P. aeruginosa*, *A. baumannii*, *K. pneumoniae*, and *P. mirabilis*) and year of collection (2002 to 2005, 2006 to 2008, and 2009 to 2011) as factors was conducted (Table 2 and Table 3). The interaction term was statistically significant, indicating that bacterial species behaved differently through the years in terms of their frequencies of isolation. In order to detect the precise source of variation, *post hoc* LSD tests comparing the means of the results were conducted (Table 2). These analyses showed that the incidence of *S. marcescens* infection or coloniza-

TABLE 1 Microbiological and molecular features of *S. marcescens* strains from this study

Hospital/location and sample no.[a]	Date of isolate collection (mo/day/yr)	Isolate source[b]	PFGE subtype[c]	Gene amplification[d]				Antimicrobial sensitivity pattern[e]
				intI1	$bla_{\text{CTX-M-2}}$	$bla_{\text{PER-2}}$	*qnrB*	
H1/PBA								
13007	08/03/2007	TA	IX	−	−	−	−	CAZ, CTX, PTZ, IMP, MER, GEN, AK, NAl, CIP, TMS
13008[f]	11/13/2007	Bd	Ib	+	+	−	−	IMP, MER
13003[f]	11/19/2007	Bd	Ib	+	+	−	+	IMP, MER
13006[f]	12/23/2007	Bd	Ib	+	+	−	−	IMP, MER
13004[f]	02/25/2008	Bd	Ib	+	+	−	+	IMP, MER
13009[f]	03/01/2008	Bd	Ib	+	+	−	−	IMP, MER
13010[f]	04/01/2008	Bd	Ib	+	+	−	−	IMP, MER
13011[f]	04/07/2008	Bd	Ib	+	+	+	−	IMP, MER
13005[f]	04/18/2008	Bd	Ib	+	+	−	+	IMP, MER
13001[f]	04/22/2008	BAL	Ib	+	+	−	−	PTZ, IMP, MER
13015	06/09/2010	BAL	NT	−	−	−	−	CAZ, CTX, PTZ, IMP, MER, GEN, AK, NAl, CIP, TMS
13014	06/30/2010	Bd	XIV	−	−	−	−	CAZ, CTX, PTZ, IMP, MER, GEN, AK, NAl, CIP, TMS
13012	07/19/2010	M-BAL	Ib	−	−	−	−	CAZ, CTX, PTZ, IMP, MER, GEN, AK, NAl, CIP, TMS
13013	07/23/2010	CF	X	+	+	−	−	PTZ, IMP, MER, GEN, AK, NAl, CIP, TMS
H2/SF								
886[f]	2002	CF	II	+	+	−	−	PTZ, IMP, MER, GEN, AK, NAl, CIP, TMS
887[f]	2002	Bd	II	+	+	−	−	PTZ, IMP, MER, GEN, AK, NAl, CIP, TMS
888[f]	2002	Bd	II	−	−	−	−	CAZ, CTX, PTZ, IMP, MER, GEN, AK, NAl, CIP, TMS
889[f]	2002	Bd	II	−	−	−	−	CAZ, CTX, PTZ IMP, MER, GEN, AK, NAl, CIP, TMS
896	2003	Bd	V	−	−	−	−	CAZ, CTX, PTZ, IMP, MER, GEN, AK, NAl, CIP, TMS
H3/PBA								
497[f]	2002	BAL	Ic	+	+	−	−	PTZ, IMP, MER, CIP, GEN, AK, NAL, TMS
H4/BA								
15	2001	BAL	XIII	−	−	−	−	CAZ, CTX, PTZ, IMP, MER, GEN, AK, NAl, CIP, TMS
H5/ER								
279	2000	Bd	VI	+	+	−	−	PTZ, IMP, MER, CIP, GEN, AK, NAL
H6/PBA								
256	1999	Bd	VII	+	+	−	−	PTZ, IMP, MER, CIP, GEN, AK, NAL, TMS
H7/BA								
102	1997	BAL	III	−	−	−	−	CAZ, CTX, PTZ, IMP, MER, GEN, AK, NAl, CIP
H8/BA								
311	2004	BAL	III	+	−	−	−	PTZ, IMP, MER, NAl, CIP
314	2005	Bd	Id	−	−	−	−	CAZ, CTX, PTZ, IMP, MER, NAl, CIP, GEN
H9/BA								
401	2005	Bd	XII	+	−	−	−	CAZ, CTX, PTZ, IMP, MER, GEN, AK, NAl, CIP
404	1998	BAL	XI	+	−	−	−	CAZ, CTX, PTZ, IMP, MER, AK, NAl, CIP, TMS
408	1998	BAL	VIII	−	−	−	−	CTX, PTZ, IMP, MER, AK, NAl, CIP
H10/BA								
2000	2006	BAL	IV	+	−	−	−	CAZ, CTX, PTZ, IMP, MER, GEN, AK, NAl, CIP
2001	2006	BAL	Ia	+	−	+	+	PTZ, IMP, MER, AK
H11/BA								
1003	2007	BAL	Ib	+	+	−	−	PTZ, IMP, MER, GEN, AK
1002	2008	BAL	IV	+	+	−	−	PTZ, IMP, MER, GEN, AK
1000	2008	BAL	Ia	−	−	−	−	CAZ, CTX, PTZ, IMP, MER, GEN, AK, NAl, CIP, TMS

[a] PBA, province of Buenos Aires; SF, province of Santa Fé; BA, Buenos Aires City; ER, Province of Entre Ríos.
[b] TA, tracheal aspirate; Bd, blood; BAL, bronchoalveolar fluid; M-BAL, mini-BAL; CF, cerebrospinal fluid.
[c] Genotype determined by pulse field gel electrophoresis (PFGE). NT, nontypeable. PFGE patterns were arbitrarily named with a roman number, with letters indicating subtypes within a cluster (patterns sharing more than 85% similarity [e.g., Ia]) (18–20).
[d] − and +, negative and positive, respectively, for gene amplification by PCR by specific primers (21–23).
[e] All the isolates were resistant for the remaining antibiotic agents. CAZ, ceftazidime; CTX, cefotaxime; PTZ, tazobactam-piperacillin; IMP, imipenem; MER, meropenem; NAL, nalidixic acid; CIP, ciprofloxacin; GEN, gentamicin; AK, gentamicin; TMS, trimethoprim-sulfamethoxazole.
[f] Strains isolated during an outbreak.

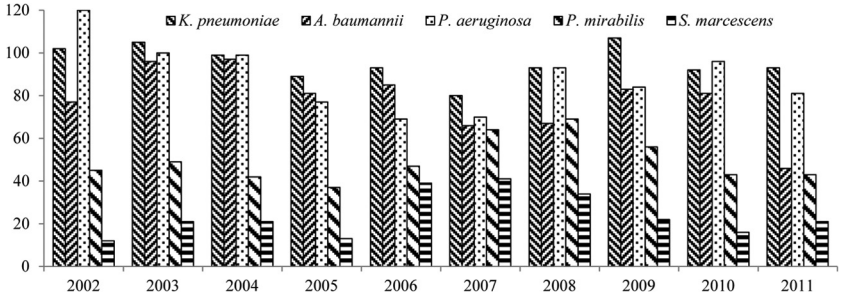

FIG 1 Isolates of *K. pneumoniae*, *A. baumanni*, *P. aeruginosa*, *P. mirabilis*, and *S. marcescens* recovered in 2002 to 2011 in hospital H1.

tion was significantly higher in the 2006-to-2008 period than in the other two periods (Fig. 2). This increase was observed after the treatment of infections caused by carbapenem-resistant *A. baumanni* isolates with colistin which at the same time produced both a significant decrease of the incidence of *P. aeruginosa* and *A. baumannii* infections from 2005 to the present and a reduction of the frequency of cases that required the administration of this antibiotic to date (Fig. 2 and 3 and Table 2). *P. mirabilis*, also a naturally colistin-resistant species, showed the same behavior as *S. marcescens*; i.e., the incidence of *P. mirabilis* was significantly greater in the 2006-to-2008 period (Table 2 and Table 3). During 2002 to 2011, only one other outbreak, due to colistin-resistant *Klebsiella pneumoniae* isolates collected in hospital H1 in 2009, was reported (29).

Microbiological investigation and end of the outbreak. The retrospective review of *S. marcescens* infections in the database of the hospital's microbiology laboratory was conducted, and an exhaustive environmental mapping of patients located in the clinical medicine area was simultaneously performed. Thirty-three surveillance cultures were done in April 2008 in order to detect putative nosocomial reservoirs of *S. marcescens*. Sampling of the environment ($n = 13$) included hospital stretchers, ventilator humidifiers, furniture, room surfaces, disinfectant solutions, and dextrose (5% solution) samples. The staff members ($n = 20$) (physicians and nurses) were screened for carriage of the organism by finger impression onto nutrient agar plates. No *S. marcescens* isolates were found from surveillance cultures. Although 41% of the *S. marcescens* isolates collected from infected patients in November 2007 and April 2008 were from blood possibly associated with previous vascular access, no positive cultures were found in catheters and additional devices. Patients colonized or infected with multidrug-resistant *S. marcescens* were kept in isolation rooms until their discharge in order to minimize cross-transmission of the microorganism. Notification and education protocols on *S. marcescens* and standard infection control procedures were reviewed together with the hospital personnel. Cleaning, reinforcement of hand washing, and disinfection procedures were reviewed and strengthened with environmental-service personnel. The implementation and persistence of these prevention measures have been effective for the eradication of outbreaks of multidrug resistant *S. marcescens* isolates since they were introduced.

TABLE 2 Two-way factorial analysis of variance applied to *A. baumannii*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, and *S. marcescens* isolates

Parameter	Value[a]				
	SS	df	MS	*F*	*P*
Intercept	219,711.8	1	219,711.8	2,022.1	0.0001
Species	33,086.8	4	8,271.7	76.1	0.0001
Yrs	201.2	2	100.6	0.9	0.405
Interaction	2,824.2	8	353.0	3.2	0.007
Error	3,803.0	35	108.7		

[a] Data represent square-root-transformed values from comparisons between categories of years (2002 to 2005, 2006 to 2008, and 2009 to 2011). SS, sum of squares; df, degree of freedom; MS, mean square; *F*, analysis of variance (ANOVA) statistics; *P*, significance level.

Molecular study of *S. marcescens* isolates from hospital H1 and other hospitals. The 9 isolates from the outbreak at hospital H1 showed the same PFGE profile and were named cluster I (Fig. 3). These isolates were compared by PFGE to other *S. marcescens* isolates belonging to well-characterized outbreak and sporadic isolates from 10 hospitals in 3 provinces of Argentina (1998 to 2010). Cluster I was highly (more than 85%) related to another isolate recovered from an outbreak at H3 (isolate 497) and from sporadic sources (isolates 314, 1000, 2001, and 1003). Strain 13007 isolated before the outbreak in hospital H1, as well as other strains isolated after the outbreak, presented an unrelated PFGE pattern (Table 1). The remaining sporadic isolates studied from various hospitals showed diverse patterns compared to those of cluster I (Fig. 3 and Table 1).

Antimicrobial resistance determinants of *S. marcescens* isolates. A different profile of susceptibility was found in strains recovered from the outbreak compared to strains isolated before and after the outbreak in hospital H1. On the one hand, most isolates that were recovered before and after the outbreak from infected patients (such as isolates 13007, 13012 13014, and 13015) were susceptible to most of the antibiotics assayed and belonged to different clusters (Table 1). On the other hand, all strains corresponding to cluster I isolated from the outbreak's source in hospitals H1 and H3 were found to carry the $bla_{\text{CTX-M-2}}$ gene which was found as part of the In132 complex class 1 integron (23). This class 1 integron possesses the gene cassette *aac(6′)-Ib*, which confers resistance to amikacin, followed by the gene cassette *aadA1*, which confers resistance to streptomycin. Isolate 13011 belonging to cluster I from hospital H1 and isolated from the outbreak was also positive for the $bla_{\text{PER-2}}$ gene, and other 4 isolates from the outbreak, isolates 13001 and 13003 to 13005, were positive for the *qnrB10* gene (Table 1). These genes were also present in the 2001 strain which was a sporadic isolate belonging to cluster I in hospital H10. Although several isolates harbored the In132::*IS*CR1::$bla_{\text{CTX-M-2}}$ complex class 1 integron, which had the *aac(6′)-Ib* gene cassette, resistance to amikacin was not detected in some

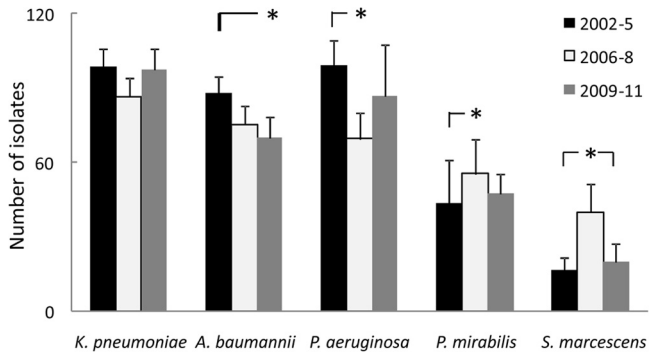

FIG 2 Isolates of the most relevant *S. marcescens* and Gram-negative bacterial species isolated from hospital H1 in 2002 to 2011. Each asterisk indicates statistically significant differences ($P < 0.05$) between the data in the column with the asterisk above and the data in the one or two columns that are linked with lines coming out of the asterisk, as determined using the statistical analysis described in detail in Table 3.

strains (e.g., 13013, 886, 887, 497, 279, 256, 1002, 1003). This result is in agreement with previous findings from our laboratory, since we identified several Gram-negative isolates possessing *aac(6′)-Ib* but not exhibiting at a phenotypic level resistance to amikacin (data not shown).

Transfer of In132::*IS*CR1::$bla_{\text{CTX-M-2}}$ and In131::IS*CR1*::*qnrB10* was assayed by biparental conjugation, and it was not possible to obtain the corresponding transconjugants in either of the two cases (data not shown). Some of the *S. marcescens* clusters corresponding to epidemiologically unrelated isolates were positive for the $bla_{\text{CTX-M-2}}$ gene, and none of the isolates tested had the $bla_{\text{SHV-like}}$, $bla_{\text{GES-like}}$, $bla_{\text{VEB-like}}$, $bla_{\text{IMP-like}}$, $bla_{\text{VIM-like}}$, or $bla_{\text{SPM-1}}$ genes.

DISCUSSION

Administration of colistin in several wards of hospital H1 since 2005 was concomitant to (i) a significant increase in the frequency since 2006 of *S. marcescens* and *P. mirabilis* isolates, both species naturally resistant to that antibiotic, (ii) an outbreak due to the rapid spread of a multidrug-resistant *S. marcescens* cluster harboring complex class 1 integrons with a high rate of mortality (40%) from November 2007 to April 2008, (iii) a significant decrease of the incidence of *P. aeruginosa* and *A. baumannii* infections since 2005, and (iv) an outbreak due to colistin-resistant *K. pneumoniae* from October 2009 to January 2010 (29). These data outline a scenario in which epidemiological patterns that include infrequent species and also multidrug-resistant strains change over the years due in part to administration of the antibiotic in hospital settings. The observed correlation between the introduction of colistin and the increase in the rates of infections by *S. marcescens* and *P. mirabilis* should be confirmed by future studies that investigate causative relationships.

Cluster I with epidemic behavior of *S. marcescens* was dispersed after 2002 in several cities from Argentina (Table 1). It was identified in 5 of 11 of the studied hospitals, being in two cases isolated from outbreaks. This cluster possessed several distinct PFGE subtypes compared to other clusters, suggesting that it has been evolving in Argentinean hospital settings for at least a decade (Fig. 1). Recently, the ability of certain Gram-negative bacilli, including *Enterobacter cloacae* and *K. pneumoniae*, to cause nosocomial out-

TABLE 3 Values of statistical significance of the comparisons between means using a LSD *post hoc* test conducted after the analysis of variance whose results are presented in Table 2[a]

Species	Yrs	*P*											
		K. pneumoniae (2002–2005)	*K. pneumoniae* (2006–2008)	*K. pneumoniae* (2009–2011)	*A. baumannii* (2002–2005)	*A. baumannii* (2006–2008)	*A. baumannii* (2009–2011)	*P. aeruginosa* (2002–2005)	*P. aeruginosa* (2006–2008)	*P. aeruginosa* (2009–2011)	*P. mirabilis* (2002–2005)	*P. mirabilis* (2006–2008)	*P. mirabilis* (2009–2011)	*S. marcescens* (2002–2005)	*S. marcescens* (2006–2008)
K. pneumoniae	2002–2005														
K. pneumoniae	2006–2008	0.21													
K. pneumoniae	2009–2011	0.86	0.32												
A. baumannii	2002–2005	0.14	0.91	0.24											
A. baumannii	2006–2008	<0.01	0.07	0.01	0.07										
A. baumannii	2009–2011	<0.01	0.04	<0.01	***0.03***	0.76									
P. aeruginosa	2002–2005	0.97	0.20	0.84	0.14	<0.01	<0.01								
P. aeruginosa	2006–2008	0.01	0.19	0.02	0.20	0.59	0.39	***0.01***							
P. aeruginosa	2009–2011	0.15	0.85	0.23	0.93	0.10	0.05	0.14	0.26						
P. mirabilis	2002–2005	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	***0.04***				
P. mirabilis	2006–2008	<0.01	<0.01	<0.01	<0.01	0.15	0.25	<0.01	0.05	<0.01	0.61	0.15			
P. mirabilis	2009–2011	<0.01	<0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	<0.01	0.51	0.01	0.28	***0.01***	
S. marcescens	2002–2005	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.72	***0.04***
S. marcescens	2006–2008	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		
S. marcescens	2009–2011	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01		

[a] Five relevant significant values are indicated in bold and italics.

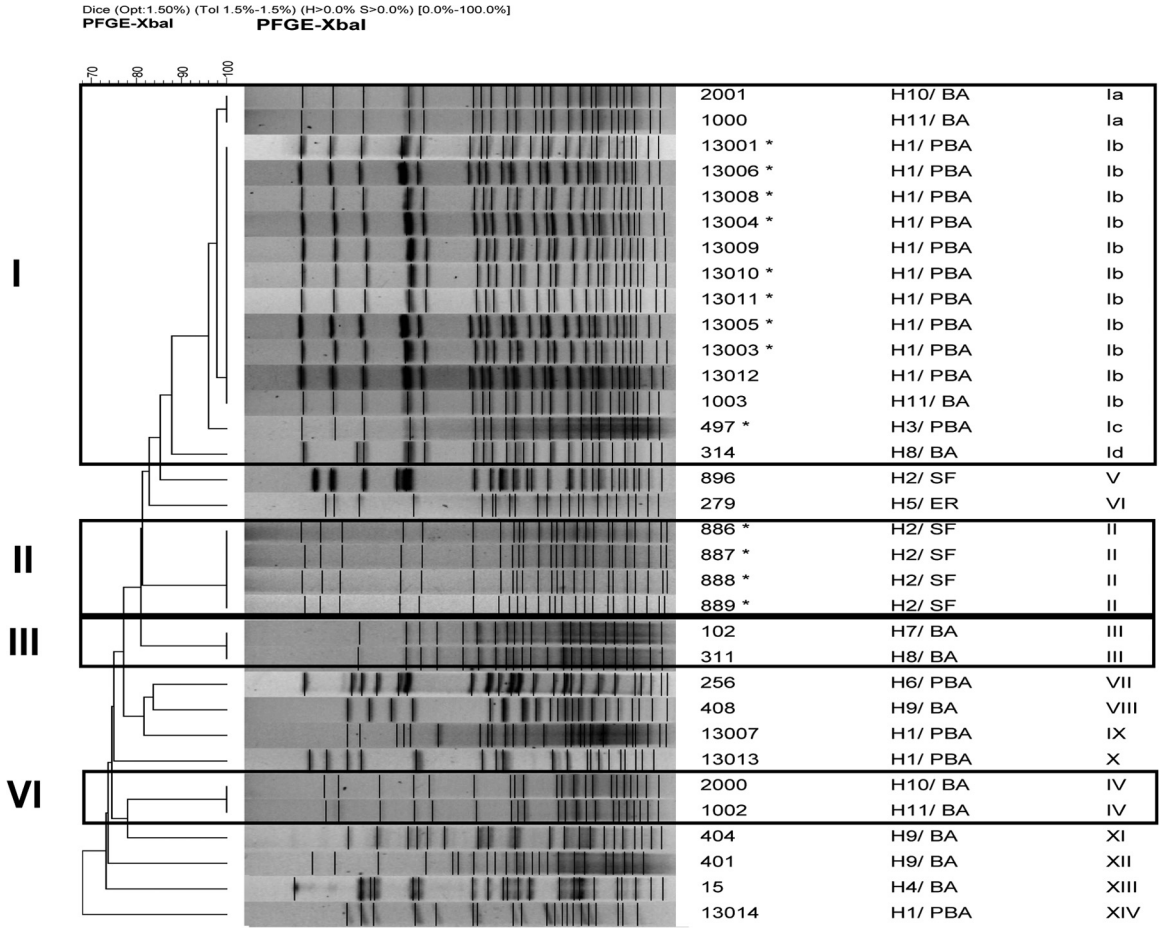

FIG 3 XbaI-PFGE dendrogram of *S. marcescens* isolates. The cutoff value for cluster delineation was 85% similarity. Isolates included in a cluster (I, II, III, or IV) are indicated with rectangular boxes. Cluster I included different fingerprints, which were denoted with letters (a, b, c, and d). *, isolates associated with the outbreak.

breaks associated with the spread of epidemic multidrug-resistant clusters carrying integrons has been documented (30–32). In the present study, the sporadic isolates of *S. marcescens* found before and after the outbreak did not possess class 1 integrons (Table 1). In contrast, strains from cluster I isolated from outbreaks in hospital H1 and H3 were found to carry the complex class 1 In132::*IS*CR1::$bla_{CTX\text{-}M\text{-}2}$ integrons. It is likely that this cluster belongs to this group of *Enterobacteriaceae* isolates that can be rapidly spread and maintained in outbreaks due, in part, to the acquisition of antimicrobial resistance properties conferred by class 1 integrons and ESBLs. In addition, the finding of $bla_{PER\text{-}2}$ and *qnrB10* in several cluster I strains isolated from the outbreak in hospital H1 supports the idea that accumulation of multiple genetic determinants in a single cell contributes to the evolution of pan-drug-resistant clusters with epidemic behavior in *S. marcescens* isolates. This process can have important clinical and therapeutic implications, as it is involved in the maintenance and spread of virulent bacterial cells in the nosocomial environment, increasing nosocomial morbidity and mortality. *S. marcescens* is a well-recognized cause of hospital-acquired infection during the three last decades, mainly in areas of risk and immunocompromised patients. Several outbreaks, more commonly in neonatal units than in adults, have been documented and were associated with a single cluster (32, 33) as well as with the combination of two or more clusters (3, 34). Here, the mean age of the patients in the clinical area of the H1 hospital was 53 years, a bit higher than what is found in the literature (32–35). Although an environmental source, mainly hospital settings and fomites, has been found in some outbreaks (13, 36), most of these events were characterized by the lack of a detectable cause (2, 34). In this study, environmental reservoirs of *S. marcescens* in the clinical medicine area were not found, probably due to the delay in collecting samples after case onset (six months, from November 2007 to April 2008).

The inception of colistin treatment in hospital H1 and the related later increase of the recovery of naturally resistant species such as *S. marcescens* and *P. mirabilis* should be taken as signals of a change in the bacterial epidemiology of the hospital settings. This resulted not only in already described outbreaks due to selection of colistin-resistant *K. pneumoniae* isolates in hospital H1 as well as in other hospitals (17, 29) but also in a rise in the incidence of isolation of infrequent species within the nosocomial environment such as *S. marcescens*. The success obtained with measures taken to control the outbreak in hospital H1 showed that it is necessary to optimize the use of colistin simultaneously with improvement of infection control to prevent the spread of species naturally resistant to colistin in order to preserve this antimicrobial agent as a clinical option.

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