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1 **Long-lasting nosocomial persistence of chlorhexidine-resistant**

2 ***Serratia marcescens* in a veterinary hospital**

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15
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19
20
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24 **Abstract**

25 Healthcare-associated infections (HAIs) are often overlooked in veterinary medicine. *Serratia*
26 *marcescens* isolates were recovered over a ten-year period from companion animals in a French
27 veterinary hospital. The pets were sampled either for diagnostic purposes or to monitor
28 colonization. A retrospective study showed that 32 *S. marcescens* isolates were identified as HAI
29 cases and a further 22 cases were associated with colonization of the surgical site. Two *S.*
30 *marcescens* lineages were responsible for two different outbreaks during the study period.
31 Chlorhexidine solution (1%) used to impregnate gauze was found to be the source of the second *S.*
32 *marcescens* outbreak and all isolates had high MIC values for chlorhexidine (MIC=128 mg/L). This
33 study reports, for the first time to our knowledge, the nosocomial spread of chlorhexidine-resistant
34 *S. marcescens* in a veterinary setting and highlights consequences of the improper use of
35 disinfectants.

36 1. Introduction

37 Companion animals can be infected or colonized by pathogenic or commensal microorganisms.
38 Some of these microorganisms potentially represent both animal and public health threats,
39 especially when multidrug-resistant bacteria are involved. They can also spread efficiently within
40 veterinary settings and cause healthcare-associated infections (HAIs) with various epidemiological
41 facets.

42
43 The consequences of HAIs in animal health include increased morbidity and/or mortality. Public
44 health issues relate not only to the possible transfer of nosocomial pathogens to healthcare staff
45 and/or other animals in contact (Wright et al., 2008), but also to the risk of spreading pathogens—
46 some of which are furthermore multidrug-resistant—beyond veterinary care facilities, including to
47 animal owners and people in contact (Wieler et al., 2011). The economic consequences of HAIs are
48 also potentially serious for the veterinary hospital involved (Benedict et al., 2008; Deschamps et al.,
49 2015).

50
51 Although little-reported, the widespread nature of HAIs in veterinary medicine may be similar to
52 that in human medicine (Stull and Weese, 2015). In addition, significant associations between
53 animal hospitalization and the carriage of multidrug-resistant bacteria in animals have been
54 demonstrated (Gibson et al., 2011; Hamilton et al., 2013). Even though viral infections should not
55 be underestimated (Deschamps et al., 2015; Stull and Weese, 2015), the etiology of nosocomial
56 outbreaks in veterinary medicine mostly involves bacteria such as *Staphylococcus*
57 *pseudintermedius*, *Enterobacterales* or *Acinetobacter baumannii* (Boerlin et al., 2001; Gronthal et
58 al., 2014; Haenni et al., 2013; Haenni et al., 2012).

60 Formerly considered a harmless saprophyte, *Serratia marcescens* has been recognized in human
61 medicine as a major opportunistic pathogen combining a propensity for HAI and antimicrobial
62 resistance. It has in particular been implicated in neonatal intensive care units (Adamson et al.,
63 2012; Zingg et al., 2017) but also in adults (Merino et al., 2016; Yoon et al., 2005). *S. marcescens*
64 has been associated with various infections, such as respiratory or urinary tract infections,
65 septicemia, meningitis, and wound infections (Hejazi and Falkiner, 1997). In veterinary medicine,
66 very few cases of nosocomial infections due to this bacterial species have been reported (Colahan et
67 al., 1984; Fox et al., 1981), and the few that have been are outdated.

68

69 A recent increase in the incidence of isolation of *S. marcescens* from animals admitted to a
70 veterinary hospital in Montpellier, France, suggested possible cases of HAI, and prompted us to
71 conduct a ten-year retrospective study in this setting. The aims of this study were to report on this
72 outbreak, characterize the bacteria isolated and identify the source of infection in order to
73 implement control measures.

74 2. Materials and methods

75 **2.1. The veterinary hospital setting.** The veterinary hospital is a private practice (Montpellier,
76 France) operating on a regional scale, mostly as a referral hospital. It provides 24/7 emergency and
77 intensive care services with a broad technical offer, including a magnetic resonance imager, a CT
78 scanner, and several operating rooms dedicated to orthopedics, neurosurgery, and soft tissues.
79 From 2009 to 2018, the activity gradually increased to reach 15,000 visits per year and 1,300
80 surgical operations (55% orthopedic). Approximately 65% of the patients were dogs and 30%
81 were cats. The antibiotic usage policy for surgery included 20 mg/kg of intravenous amoxicillin 30
82 minutes prior to incision, repeated every 90 minutes. For orthopedic operations, 12.5 mg/kg
83 amoxicillin/clavulanic acid was continued *per os* twice a day for 6 days. In the event of confirmed
84 infection, specific antibiotic treatment was prescribed depending on the bacterial species and
85 antimicrobial susceptibility profile.

86
87 **2.2. Epidemiological investigations.** The study was conducted from September 2009 to
88 September 2018 on 66 animals from which a *S. marcescens* isolate was recovered. Based on the
89 definition proposed by the Center for Disease Control (CDC;
90 <https://www.cdc.gov/hai/ssi/ssi.html>), HAI were defined according to the type of infection and the
91 onset of the infection. Cases of surgical site infection included all kinds of infections that occurred
92 after surgery in the part of the body where surgery took place, this included infections involving
93 organs, joints, or implanted material but also superficial infections involving the skin or
94 subcutaneous tissues. A case of *S. marcescens* HAI was defined when the infection's onset ranged
95 from 48 hours after admission up to 30 days after hospitalization (Stull and Weese, 2015).
96 Infections of the surgical site were defined as occurring within 30 days of surgery or in the year
97 following surgery in the case of a surgical implant or prosthesis (Haut Conseil de la Santé
98 Publique, 2020). When *S. marcescens* was isolated from a surgical site without further clinical

99 signs of infection, it was recorded as colonization. Six additional *S. marcescens* isolates were also
100 recovered from 37 environmental swabs (surgical rooms, kennels, taps, sinks, alcohol or
101 chlorhexidine-impregnated compresses, disinfecting sprays endoscopy probes and gas anesthesia
102 tubes) within the hospital in January 2019 (n=3/32) and April 2019 (n=3/5).

103
104 **2.3. Bacterial samples and identification.** All the bacteriological samples were sent to the same
105 veterinary laboratory. The specimens for bacterial culture originated from different sources as
106 mentioned above, i.e. from infected and non-infected surgical sites and the environment. Swabs
107 were plated directly on selective (Drigalski and CNA Columbia agar – Biomérieux, France) and
108 non-selective media (Columbia agar – Oxoid, France), and bacteria were cultured aerobically. On
109 the same time, an enrichment step was systematically performed in tryptic soy broth (Biokar
110 diagnostics, Pantin, France), which was only used in the absence of growth by direct plating.
111 Identification was performed using API20E galleries (Biomérieux, France), and confirmed using a
112 MALDI TOF VITEK MS Version 3.0 (bioMérieux, Marcy L'Etoile, France).

113
114 **2.4. Susceptibility testing.** Antimicrobial susceptibility was tested by disk diffusion on Mueller-
115 Hinton agar (BioRad, Marne-la-Coquette, France) in accordance with the guidelines and clinical
116 breakpoints of the Antibiogram Committee of the French Society for Microbiology
117 (<https://www.sfm-microbiologie.org>). The following disks of human and/or veterinary interest
118 were tested: amoxicillin, amoxicillin + clavulanic acid, cefalotin, cefuroxime, cefotaxime,
119 ceftiofur, ertapenem, ceftazidime, ceftiofur, ceftazidime, ceftiofur, ceftiofur, ceftiofur, ceftiofur,
120 kanamycin, amikacin, apramycin, gentamicin, tobramycin, netilmicin, chloramphenicol,
121 florfenicol, tetracycline, colistin, sulfonamides, trimethoprim, nalidixic acid, enrofloxacin, and
122 ofloxacin. *E. coli* ATCC 25922 was used as a quality control. The minimum inhibitory
123 concentration (MIC) of chlorhexidine was determined for all isolates. In brief, chlorhexidine with

124 a purity $\geq 99.5\%$ (Sigma, USA) was dissolved, diluted, then incorporated into Mueller-Hinton agar
125 in serial twofold concentrations from 0.125 to 1024 $\mu\text{g/ml}$. Bacteria were grown at 37°C for 6
126 hours under shaking conditions in Mueller-Hinton broth. All isolates were tested in duplicate. The
127 culture was deposited on the agar plates with an automatic inoculum replicator to reach a final
128 inoculum of approximately 1 to 2.10^4 CFU, as recommended by the CLSI M07 method (CLSI,
129 2018). Six *S. marcescens* from unrelated epidemiological contexts were used as controls. These
130 strains were collected from captive (one gazelle, one bighorn and two clawed frogs) or wild-life
131 animals (one hare, one hedgehog) that never visited the clinic, and the probability to have the
132 same clone circulating in such diverse contexts is very low. After 16-24h incubation of the plates
133 at 37°C , the MIC was recorded as the lowest chlorhexidine concentration that completely inhibited
134 growth.

135

136 **2.5. Molecular typing of the isolates.** Pulsed-field gel electrophoresis (PFGE) patterns were
137 obtained after digestion of the DNA with the *XbaI* restriction enzyme. The following
138 electrophoresis conditions were used: voltage at 6 V/cm, run time 24 h, initial switch time of 10 s,
139 final switch time of 60 s, angle of 120° . DNA fingerprints were analyzed using the Dice
140 correlation coefficient, with tolerance and optimization set at 0.5 and 1% respectively
141 (BioNumerics, Ghent, Belgium).

142

143 **2.6. Whole genome sequencing.** The genomic DNA of five selected isolates (Table S1) was
144 extracted from an overnight culture using the NucleoSpin Microbial DNA (Macherey Nagel,
145 Germany). The whole genome was sequenced using NovaSeq technology (Illumina). After
146 Trimmomatic cleaning, *de novo* assembly was performed using Shovill (version 0.9.0). Virulence
147 factors and resistance genes were sought from the assembled genomes using VirFinder, ResFinder
148 (CGE, <http://www.genomicepidemiology.org/>), and CARD (<https://card.mcmaster.ca/>) databases.

149 SNPs were determined using CSI Phylogeny 1.4 (online tool available on the CGE website) set at
150 its default parameters. The whole genome shotgun project was deposited in
151 DDBJ/EMBL/GenBank under accession number PRJNA607033.

3. Results

3.1. Description of the cases

Sixty-six *S. marcescens* isolates of animal origin were included in the study, mostly recovered from dogs (n=45) but also from cats (n=19) and rabbits (n=2). The monthly incidence of *S. marcescens* isolation (Figure 1A) showed two clusters, from September to December 2009 (n=8) and from March 2014 to September 2018 (n=58).

S. marcescens isolation mainly occurred in two situations. The first one referred to diagnostic swabs (n=35) performed in case of a suspected or proven case of infection. These swabs were mostly collected after orthopedic (bones – osteosynthesis and joints – tibial plateau leveling osteotomy, arthrodesis) and soft tissue surgery (total ear canal ablation, lateral bulla ostectomy, abscess, subcutaneous ureteral bypass). The second one referred to monitoring swabs (n=31) taken at the end of surgery to be sure that no important pathogen was present at the surface of the scar (Table 1). Among the 35 diagnostic isolates, 23 *S. marcescens* samples were categorized as HAI cases (infection onset >48 hours after surgery), whereas HAI was excluded for eight isolates or could not be confirmed for four isolates. Among the 31 monitoring swabs, 22 were categorized as colonization since the animal did not develop any subsequent infection, whereas nine were categorized as HAI since the animal came back to the clinic with infection signs. Consequently, 32 (23+9) cases were classified as HAI, eight as non-HAI, 22 as colonization and four as undetermined (Table 1).

HAI cases were mostly associated with bone/joint (24/32, 75.0%) or inner ear infections (15/32, 15.6%), whereas only one case was associated with a urinary tract infection after catheterization. The time between admission and detection of the infection spanned from 3 to 307 days (median=14 days). Colonization was mostly observed after bone surgery (8/22), total ear canal ablation (6/22), but also urinary (2/22), subcutaneous/muscular (3/22), abdominal (2/22) or thoracic (1/22) surgery.

176 Most isolates were cultivated without broth enrichment for both HAI (59.4%, 19/32) and
177 colonization cases (63.6%, 14/22). A large proportion of the isolates (53.7%, 29/54) were obtained
178 in pure culture, while 25 cases showed the co-contamination of *S. marcescens* with other bacterial
179 species, especially staphylococci (n=13).

180

181 **3.2. Antibiotic treatment and antibiotic susceptibility**

182 All 66 *S. marcescens* isolates showed the intrinsic antibiotic resistance profile of this bacterial
183 species, namely resistance to narrow-spectrum penicillins, amoxicillin/clavulanic acid and
184 cephalosporins, macrolides, tetracyclines, and colistin. Information about antibiotic treatments was
185 available for 26 HAI cases, showing that most patients (20/26, 76.9%) had been given amoxicillin
186 or amoxicillin/clavulanic acid after surgery while six others had been treated with marbofloxacin
187 (6/26, 23.1%). Two animals with an infection at the site of abdominal surgery had not been given
188 any antibiotics after the surgery. Considering the evidence of *S. marcescens* infection for those
189 patients, antibiotic treatment was changed for fluoroquinolones in 70% (14/20) of the animals
190 receiving penicillins. Antibiotic treatments were similar for colonized individuals, since 68.2%
191 (15/22) of the animals had been treated with amoxicillin/clavulanic acid while the others had
192 received cefalexin (1/22), marbofloxacin (5/22) or trimethoprim/sulfonamides (1/22). Similarly, the
193 antibiotic treatment of most of the animals treated with penicillins (9/15, 60%) was also changed to
194 fluoroquinolones. The symptomatology evolved favorably for 72.7% (16/22) of the animals after
195 medical care, while complications were observed for 27.3% (6/22) of them, leading to long-term
196 infections, new operations or amputation. No infection-related death was observed and no
197 euthanasia was performed.

198

199 **3.3. Epidemiological investigations**

200 In order to find the source of the animal infection/colonization with *S. marcescens*, 32
201 environmental samples were collected in January 2019. Interestingly, all *S. marcescens* was
202 recovered from a plastic storage container in which the 1% chlorhexidine solution and pre-
203 impregnated gauze (n=3/32 environmental samples) dedicated to the orthopedic and soft tissue
204 operating rooms were kept. In April 2019, a second environmental sampling session (n=5) focusing
205 on the same diluted chlorhexidine solution container and two examination rooms again proved the
206 presence of *S. marcescens* (n=3/5) in the chlorhexidine container only.

207 The *S. marcescens* samples found in the chlorhexidine solution presented an MIC of chlorhexidine
208 of 128 mg/L. MICs of six *S. marcescens* collected from other animal species and from unrelated
209 epidemiological contexts ranged from 16 to 32 mg/L. MIC values were further determined for all *S.*
210 *marcescens* samples isolated either from colonization (n=22) or HAI (n=32) cases. The MIC values
211 of chlorhexidine for all 54 isolates ranged from 64 to 128 mg/L.

212

213 **3.4. Clonality**

214 PFGE analyses were conducted on the 66 *S. marcescens* samples of animal origin and the six *S.*
215 *marcescens* samples found in the chlorhexidine solution. Figure 1B and Figure 2 show that all eight
216 HAI isolates from the first outbreak (2009) shared PFGE profile 4. Outside the first outbreak, this
217 profile was also identified once in a cat colonized in 2017 (see below). Among the 24 HAI case
218 isolates from the second outbreak (2014-2018), 20 corresponded to PFGE profile 2, two were
219 smearing profiles and two could not be studied. PFGE profile 2 was also over-represented among
220 colonized surgical sites (18/22 cases), while the remaining four isolates corresponded to a smearing
221 profile (n=1), PFGE profile 4 (n=1), or could not be investigated using PFGE. All six isolates
222 recovered from the chlorhexidine-impregnated gauze tub in 2019 also matched PFGE profile 2.
223 Only three isolates with a diverging PFGE profile (profiles 1, 3, and 5) were observed, all of which

224 were from animals that did not have an HAI (one cat and the only two rabbits included in this
225 study).

226 To obtain further information on the clonality of *S. marcescens* isolates of identical PFGE profiles,
227 WGS was performed on five selected isolates corresponding to PFGE profile 4 (#50986, #51034)
228 and PFGE profile 2 (#50995, #51745, #51748). Isolate #51748 corresponding to PFGE profile 2)
229 originated from the chlorhexidine gauze. SNP-based phylogeny revealed 100% identity between
230 bacterial genomes related to the same PFGE profile, and isolates corresponding to PFGE profile 4
231 also clearly diverged from those of PFGE profile 2. These data confirmed the spread of two
232 different nosocomial *S. marcescens* lineages in the veterinary setting over the 10-year period and
233 revealed that the chlorhexidine container used for impregnating gauze was the most probable source
234 of the second *S. marcescens* outbreak.

235 In addition to phylogenetic information, WGS data were in accordance with phenotypic data and
236 showed that no known acquired genes conferring resistance to antibiotics were present in the
237 genomes. Of note, all sequenced isolates had a KpnF-like protein in their genome. In *Klebsiella*
238 *pneumoniae*, KpnEF is an efflux pump, and KpnEF mutants are known to be more susceptible to
239 hospital disinfectants such as chlorhexidine (Srinivasan and Rajamohan, 2013).

240

241 **3.5. Infection control measures**

242 All the plastic containers used to impregnate gauze with chlorhexidine were then eradicated from
243 the hospital. Importantly, gauze was no longer placed in a container to soak up diluted
244 chlorhexidine solution for antiseptis of surgical sites. New procedures for preparing the skin of
245 animals about to undergo surgery were set up. The antiseptis of skin for catheter placement and
246 urinary catheterization now relies on sterile gauze impregnated with chlorhexidine only one minute
247 before antiseptis in a small, disposable sterile tray. At the time of writing, *S. marcescens* appears to
248 have been eradicated from the hospital.

249 4. Discussion

250 This study describes for the first time the long-term occurrence of HAI due to *S. marcescens* in a
251 veterinary hospital. Such large outbreaks have already been reported in human medicine, especially
252 in neonatal intensive care units (Adamson et al., 2012; Casolari et al., 2013). Casolari *et al.* thus
253 described two long-term outbreaks in a 10-year observational study in Italy that were due to
254 multiple clones, most probably attributable to the recurrent introduction of new *S. marcescens*
255 isolates in the intensive care unit. In most reported outbreaks in human medicine, the reservoir and
256 mode of transmission remains unknown (Gastmeier, 2014). Nevertheless, the multiple introductions
257 of *S. marcescens* isolates through colonized and infected patients may represent the most frequent
258 source of infections, followed by hand-to-hand transmission inside hospital settings (Byrne et al.,
259 2001; de Vries et al., 2006). In our study, only two lineages were identified in 53/66 cases. The first
260 lineage (PFGE profile 4) was found in eight isolates in 2009, but was then only found in one cat in
261 2017. PFGE profile 2 was first identified in 2014 and persisted until 2019. *S. marcescens* was not
262 detected between 2009 and 2014 most likely due to the veterinary hospital's relocation to new
263 premises at the end of 2009. The first outbreak was stopped very quickly after its onset, and it took
264 4 years until the second outbreak was observed, which lasted over 5 years.

265
266 A large environmental sampling campaign in 2019 identified the reservoir of *S. marcescens* in the
267 second outbreak as the gauzes impregnated with 1% chlorhexidine solution kept in a closed plastic
268 container for several months. Unfortunately, no environmental samples had been taken in 2009, so
269 the source of the first outbreak remains undetermined. The PFGE profile 4 responsible for the first
270 outbreak in 2009 was only identified once during the second outbreak, from one cat in 2017. This
271 clone may have been sporadically re-introduced in the clinic, either by the animal itself or through
272 an external and undetermined source. This raises the question of the population structure of *S.*

273 *marcescens* circulating outside the clinic, which remains to be studied, and the possible
274 predominance of specific clones.

275

276 This is the first report of veterinary infections due to contaminated chlorhexidine, even though such
277 disinfectant-associated infections have already been reported in humans (Weber et al., 2007).
278 Chlorhexidine is one of the most widely-used biocides for hand hygiene, surgical site disinfection
279 and topical treatments in veterinary medicine (McDonnell and Russell, 1999). Several nosocomial
280 outbreaks have resulted from inadequate skin antisepsis or disinfection of medical devices or
281 environmental surfaces (Weber et al., 2007), including mistakes in the dilution process, inadequate
282 disinfection (duration, contact) or the use of contaminated disinfectants. Here, the mode of
283 transmission was undoubtedly the direct inoculation of the bacteria onto the surgical site during the
284 skin disinfection procedure. This is corroborated by the fact that the same PFGE profile 2 was
285 detected in nearly all the samples either from post-operative infections or to check colonization of
286 the surgical site. In most cases, other bacterial species (mostly staphylococci) were also isolated in
287 addition to *S. marcescens*. This raises the question of possible co-infection or co-colonization of the
288 sampling sites. In human medicine, Casolari et al. (2013) observed an increase in simultaneous
289 colonization by ESBL-producing bacteria during the outbreak, which may represent a risk of
290 horizontal gene transfer to *S. marcescens* isolates (Casolari et al., 2013).

291

292 All the *S. marcescens* isolates in our study were resistant to chlorhexidine (MIC values of 128
293 mg/L), as previously reported (Marrie and Costerton, 1981; Okuda et al., 1984), and presented a
294 KpnF-like protein in their genome. Whether this protein, or its over-expression, is responsible for
295 the increase in the MIC of chlorhexidine in *S. marcescens* remains to be studied. While
296 chlorhexidine MICs (128mg/L) *in vitro* are lower than the actual chlorhexidine concentration in the

297 disinfectant solution (1%), we might hypothesize that the *in vivo* tolerance is higher and/or that such
298 non-susceptibility to disinfectants facilitates biofilm formation.

299 Infections caused by *S. marcescens* can be difficult to treat because of its intrinsic resistance to a
300 variety of antibiotics, including narrow-spectrum penicillins and cephalosporins, cefuroxime,
301 cephamycins, macrolides, tetracycline, nitrofurantoin, and colistin (Stock et al., 2003). In this
302 context, an additional resistance to disinfectants may pose serious treatment challenges, since
303 antiseptics are often used to clean contaminated sites and lower the bacterial load in order to
304 optimize the effect of the antibiotic therapy. In veterinary medicine, *S. marcescens* is still mostly
305 susceptible to antibiotics, yet many human clinical isolates now demonstrate multiple antimicrobial
306 resistances to critically important antibiotics (Cristina et al., 2019). According to the antibiotic
307 policy at the time of this study, most animals were given antibiotics, in particular amoxicillin or
308 amoxicillin/clavulanic acid. In line with a prudent use of antibiotics, it is of utmost importance to
309 distinguish infections—when prompt therapy is required—from colonization—when the use of
310 antibiotics is clearly contraindicated.

311

312 A policy of frequent bacteriological checks of the surgical wound was instigated in the veterinary
313 hospital at the onset of the second epidemic cluster. Monitoring swabs were carried out in particular
314 for difficult surgical operations. This policy enabled antibiotic treatment to be re-evaluated when
315 needed, and any *S. marcescens* epidemic to be detected through its recurrent isolation. The
316 surveillance of HAI in veterinary hospitals is the key to providing information for prevention and
317 action. Key elements of early HAI identification include (1) a surveillance program tailored to the
318 risks and needs of the veterinary practice and (2) routine use of diagnostic cultures and
319 susceptibility data to establish practice-specific baseline levels of pathogen prevalence and
320 antimicrobial resistance so as to detect changes (Stull and Weese, 2015). Even though some authors
321 have reported baseline rates for HAI in small animal hospitals (Ruple-Czerniak et al., 2013), most

322 reports in veterinary medicine have addressed major epidemics, often with multidrug-resistant
323 bacteria which can be more easily identified as the cause of a nosocomial infection (Gronthal et al.,
324 2014; Haenni et al., 2013; Haenni et al., 2012). The contrast between the frequent reporting of
325 nosocomial infections due to *S. marcescens* in human medicine and the paucity of reports in
326 veterinary medicine may be due to a higher incidence of these infections in humans, particularly in
327 the high-risk neonate population. Indeed, the level of sophistication of care, the more frequent use
328 of specific invasive procedures (e.g. assisted ventilation) and the length of stay appear to be greater
329 risk factors in human settings. On the other hand, frequent handling of animals, hygiene and other
330 constraints of veterinary practice (cage rest, animals' fur, etc.) and the delay in the prevention and
331 control of these infections are probably risk factors specific to the veterinary environment (Morley,
332 2013; Stull and Weese, 2015). As the levels of care, size and organization of facilities evolve
333 gradually, veterinary practitioners need to be increasingly aware of these infections.

334 **5. Conclusion**

335 This study reports for the first time a long-lasting outbreak of chlorhexidine-resistant *S. marcescens*
336 in a veterinary hospital. The origin of the contamination was traced back to gauze soaked in 1%
337 chlorhexidine solution, used to disinfect the surgical site. The outbreak was detected mainly because
338 veterinarians took swabs of the surgical sites of complicated surgical operations. No further *S.*
339 *marcescens* infection or colonization has been discovered since disinfection procedures were
340 modified.

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345

346 **Conflict of Interest Statement**

347 The authors declare that this research was conducted in the absence of any commercial or financial
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349

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353

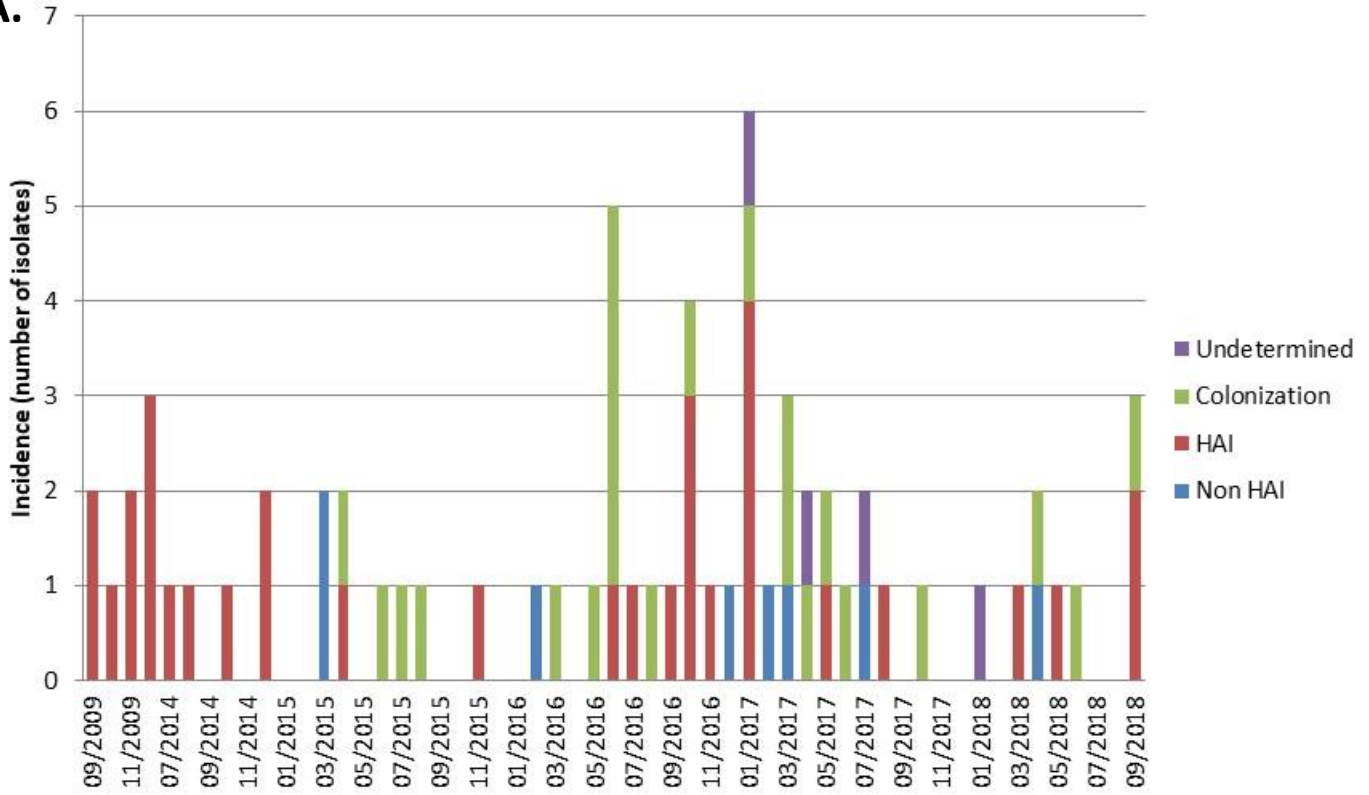
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444

Figure 1. Monthly incidence of *Serratia marcescens* isolation (n=66) from September 2009 to September 2018 related to HAI cases (A) or to PFGE profiles (B).

A.



B.

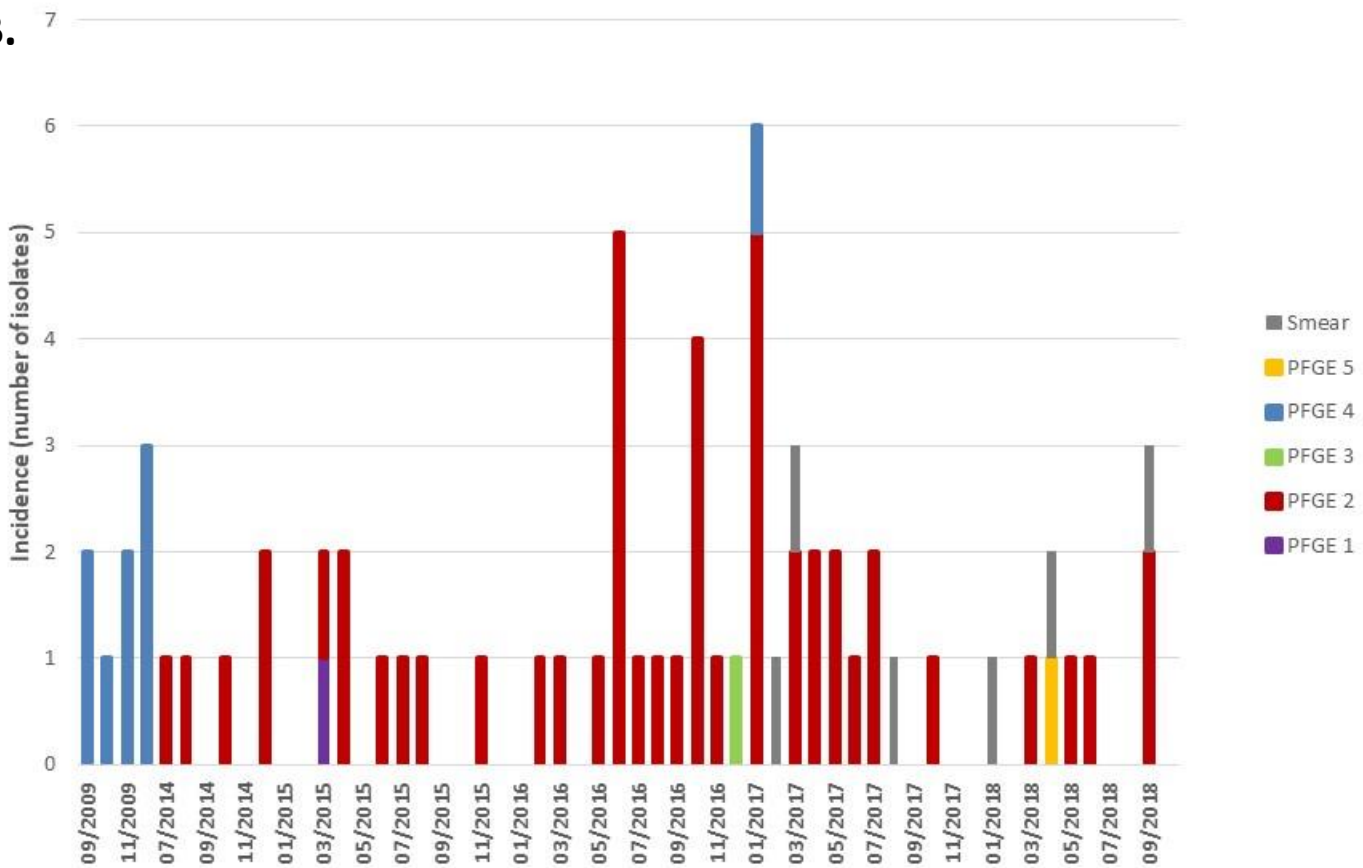


Figure 2. PFGE profiles of selected *Serratia marcescens* isolates from animals and the veterinary hospital environment. One representative isolate per PFGE profile and per year was included. The two isolates collected in 2019 are environmental isolates.

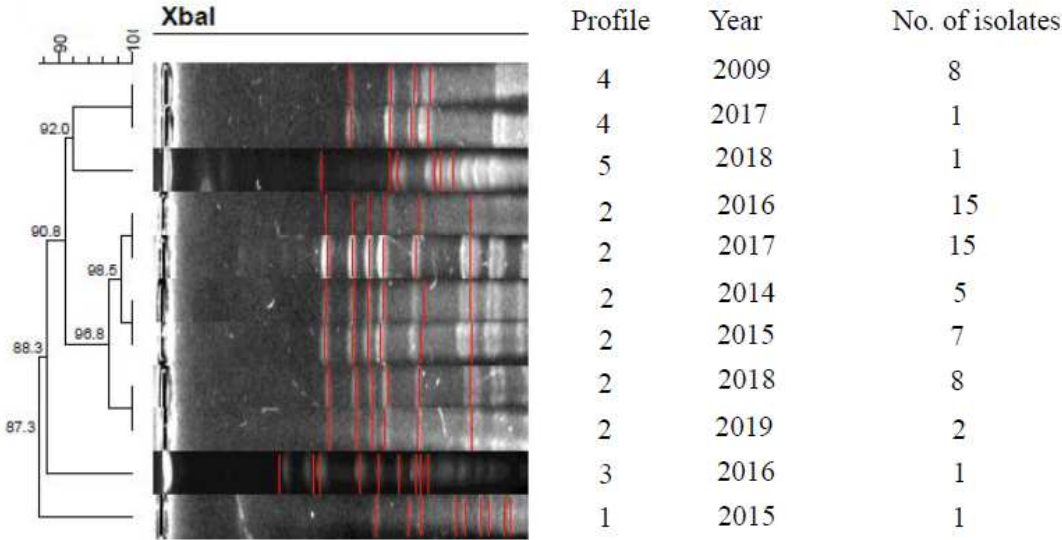


Table 1. Healthcare-associated infections (HAIs), non-HAI and colonization cases among the 66 *Serratia marcescens* isolates.

Status	Dog (n=45)	Cat (n=19)	Rabbit (n=2)	Total (n=66)
<i>Infection cases</i>				
HAI ¹	25	7	0	32
non-HAI	3 ²	3	2 ³	8
Undetermined ⁴	4	0	0	4
<i>Colonization</i>	13	9	0	22

¹ Cases of infection were considered to be HAIs when their onset was observed > 48 hours after surgery.

² One canine isolate collected in March 2015 belonged to the PFGE clone 1.

³ The two rabbits isolates belonged to PFGE clones 3 and 5, respectively.

⁴ "undetermined" corresponds to cases where the information was too scarce to determine the status