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1 **Investigation of *Serratia marcescens* surgical site infection outbreak associated with**
2 **peroperative ultrasonography probe**
3

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22 **Running title :** *Serratia marcescens* outbreak
23

24 **Abstract**

25 Early postoperative infections due to *Serratia marcescens* have been reported by both clinicians
26 and microbiologists in our teaching hospital. Here, we present an interlinked clinical,
27 epidemiological, environmental and genomic investigation of this outbreak due to a T-shaped
28 intraoperative probe contaminated by *S. marcescens* used during peroperative ultrasonography in
29 laparoscopic liver resection.

30

31 **Introduction**

32 *Serratia marcescens* is an opportunistic pathogen that can cause a wide variety of
33 healthcare-associated infections, such as respiratory and urinary tract infections, meningitis,
34 bacteraemia and surgical site infections (SSIs), particularly in settings such as intensive care
35 units (ICUs)¹⁻². This Gram-negative bacteria can easily spread in the environment and
36 contaminate medical equipment¹⁻², drugs¹⁻³, antiseptic solutions⁴, or the hands of caregivers¹.
37 Investigations usually find, as a surgical environment reservoir, razors^{5,6}, detergent-disinfectant
38 solutions⁷ or saline solutes⁸. In August 2018, four SSIs due to *S. marcescens* occurred in a
39 digestive surgery ward in a French teaching hospital. This led to extended investigations in order
40 to identify the origin of the outbreak.

41

42 **Methods**

43 This study took place in the teaching hospital of Caen, a 1,410-bed hospital located in
44 Normandy, France. On 6 August 2018, a fourth spatiotemporal case of *S. marcescens* SSI
45 appeared in the digestive surgery ward. Furthermore, the clinical strains displayed the same
46 antibiotic resistance profile. As a consequence, the hygiene and infection control department of
47 the hospital was alerted on 7 August 2018 by surgeons, microbiologists and intensive care
48 seniors; this led to the immediate closure of the operating room. To screen unnoticed cases since
49 the beginning of 2018, retrospective investigations were performed. Information was collected
50 from the acts traceability booklet and medical files, as well as coding data transmitted by the
51 hospital archive department, the microbiology laboratory and the hospital pharmacy. Cases were
52 defined as follows: any patient who had undergone digestive surgery in the incriminated
53 operating room, especially a hepatic tumour resection by radiofrequency or by hepatectomy

54 (with intraoperative ultrasound guidance) and who had a microbiological sample positive for *S.*
55 *marcescens*. The National Nosocomial Infections Surveillance (NNIS) risk index was calculated
56 for cases.⁹ Environmental samples were performed in the operating room, including airlock by
57 taking air samples (impact biocollector) and surface samples (contact agar plates), in order to
58 find the reservoir of *S. marcescens*. Isolated strains were identified using the MALDI-TOF mass
59 spectrometry method (Bruker®, Germany). Antimicrobial susceptibility was tested against a large
60 panel of antibiotics according to French and European guidelines¹⁰ for all *S. marcescens* strains,
61 clinical and environmental, isolated throughout this investigation, as well as two supplementary
62 not-linked strains. Moreover, these strains were subjected to whole-genome sequencing (WGS)
63 using a NextSeq 500 platform (Illumina, USA), generating 150 bp paired-end reads. Genomes
64 were *de novo* assembled using SPAdes v 3.12 software. Every genome is available on the NCBI
65 BioProject: PRJNA648275. All known acquired antibiotic resistance genes were screened thanks
66 to the Resfinder database (www.cge.dk). Furthermore, point mutations were searched in order to
67 detect alterations in the quinolone resistance-determining regions (QRDRs). Reads were mapped
68 against the reference genome *S. marcescens* subsp. *marcescens* Db11 (Nucleotide accession
69 number: NZ_HG326223) using the Burrows-Wheeler Aligner, and single nucleotide
70 polymorphisms (SNPs) were determined in order to evaluate the distance between all the
71 sequenced strains using mpileup, as previously described.¹⁰

72

73 **Results**

74 A total of eight patients suffered from SSIs caused by *S. marcescens* between 5 April and
75 7 August 2018 in the same digestive surgery ward. These patients underwent hepatectomy with
76 intraoperative ultrasound guidance, with four of them undergoing tumour resection by

77 radiofrequency. All eight cases had an early deep SSI (between 0 and 11 days after surgery), and
78 all but one case received adapted antibiotic treatment for 11 to 42 days. Three cases required
79 surgical revision to control the infection (see patients characteristics in Table 1). In all cases, the
80 drug used for preoperative surgical antibiotic prophylaxis and method of administration were in
81 accordance with *in situ* and French Society of Anaesthesia and Intensive Care Medicine
82 guidelines, which consists of 2 g cefazolin injection [<https://sfar.org/>]. The NNIS score was
83 variable among patients: 0 (n = 3), 1 (n = 4) and 2 (n = 1). The highest score corresponded to the
84 index case who suffered from deterioration in his general status and potentially contaminated
85 surgery. His biliary tract infection (caused by *S. marcescens*) was responsible for immediate
86 postoperative septic shock, which was treated with a combination of cefepime and gentamicin
87 for 25 days.

88 The investigation of the eight cases revealed two common points: i) the operating room
89 and ii) the use of a unique T-shaped intraoperative probe 8816 (BK Ultrasound, Peabody,
90 Massachusetts). The operating room was closed, and radiofrequency material was retired on 7
91 August 2018, whereas the ultrasound probe was sequestered three days after the alert. In fact,
92 time was needed to analyse 67 environmental samples, including surgical material samples
93 (radiofrequency material and T-shaped probes), medicine fridges, scopes, room surfaces (floors,
94 walls, materials, trolleys, operation tables, cables, scyalitic lamps, and cans), detergent-
95 disinfectant and antiseptic solutions, syringe drivers, ultrasound scanners, and computer
96 keyboards. Surgical interventions were performed by several surgical teams; hence, no *S.*
97 *marcescens* carriage testing was performed on caregivers. First, none of the 67 samples were
98 positive for *S. marcescens*. A new inspection was performed on the ultrasound probe by the
99 hygiene microbiologist. It turned out that the sleeve that allowed for the seal between the cable

100 and body of the probe was unstuck, facilitating the probable formation of a reservoir. In addition,
101 an analysis of the local practices highlighted two major non-compliances. First, the probe was
102 used intraoperatively without a single-use sterile protective sheath, and second, the maintenance
103 carried out after its use was not in conformity (absence of terminal disinfection after cleaning).
104 Thanks to these observations, new samples were taken by modifying the sampling technique in
105 pre-adding a step of sterile water immersion/injection of/in the probe, in particular in the sleeve.
106 Doing so, a *S. marcescens* strain was isolated and displayed the same pattern of antibiotic
107 resistance as those isolated from the eight patients. All these epidemic strains harboured the
108 chromosomal *AmpC bla-SRT-2* and *aac(6')-Ic* genes, conferring resistance to most penicillins,
109 including cefazolin and amikacin, respectively. Epidemic strains also carried the *bla-TEM* gene,
110 coding a penicillinase; aminoglycoside *aadA1* gene, conferring resistance to streptomycin and
111 spectinomycin; and *dfrA1* gene, leading to resistance to trimethoprim and co-trimoxazole.
112 QRDRs were chromosomally substituted in GyrA (Ser83Ile and Asp87Asn) and ParC (Ser80Ile),
113 conferring high-level resistance to fluoroquinolones through three amino acid changes. Epidemic
114 strains remained susceptible to key antibiotics for *S. marcescens* treatment (i.e., third-generation
115 cephalosporins or carbapenem). *In silico* analyses confirmed that all eight clinical strains were
116 closely related to the probe strain, with an average of distance of 9 SNPs (min: 1, max: 24) inside
117 the cluster, while a distance 87 000 SNPs split the epidemic strains with two not-linked strains
118 (see Supplemental Figure).

119 In total, the incriminated T-shape probe was used in nine interventions between the index
120 case and its withdrawal. Among them, eight patients presented with SSIs caused by *S.*
121 *marcescens*. In the only patient who did not contract an SSI, the contaminated probe was used
122 with a very short contact time. Regarding surgical procedures, numerous non-compliances were

123 uncovered, such as the absence of biocleaning and upkeep traceability for radiofrequency
124 material, T-shape probes and fridges, as well as the absence of the single-use sheath of the probe
125 during ultrasonography.

126 Not one case of *S. marcescens* SSI has been reported since the end of this investigation
127 and corrective measures have been taken, such as medical device vigilance, staff formation and
128 procedure adjustments in biocleaning and traceability and the use of the probe sheath during
129 deep surgical operations.

130

131 **Discussion**

132 This *S. marcescens* outbreak investigation revealed eight postoperative infections due to
133 the use of a contaminated T-shaped intraoperative probe. During ultrasonography, the probe was
134 used in direct and long contact with tissues without a single-use sheath. These defaults could
135 explain a brief five day-mean elapsed time between the operation and the first signs of infection.
136 This delay is shorter than that described in other publications.^{11,12} Interestingly, our investigation
137 disentangled this epidemic episode by detecting all patients (including the index case), by
138 identifying the cause of the SSI and the mechanism related to the formation of the reservoir. No
139 similar example was found in the literature. Indeed, in most of studies referring to *S. marcescens*
140 SSI outbreaks, a strain reservoir was epidemiologically suspected, rarely confirmed, or the
141 number of contaminated patients were estimated.¹⁻¹² This investigation also highlighted that the
142 sole use of contact agar plates was not sufficient to find *S. marcescens* on the probe. If a specific
143 device is suspected to be infected, we recommend the use of the technique presented in this
144 report, namely immersion of the device in sterile water and/or injection of sterile water into
145 unreachable nooks. Furthermore, WGS is a highly discriminative tool widely used in large scale

146 epidemiologic investigations^{6,10}, and we showed through our work that it is valuable to ascertain
147 our hypothesis of contamination.

148 Our investigation raised several questions concerning care practices¹³. It must be
149 emphasized that high surgical activity leaves little time for the caregivers: i) to allow a good
150 check-up of the equipment and a realization of an adapted upkeep and ii) to get practical training
151 courses and to master the procedure. In addition, the use of the ultrasound probe without a sheath
152 is a common practice by surgeons to obtain a better surgical view, but it is not without risks in
153 terms of health-associated infections. Patients contamination by the T-shaped probe could
154 depend on both bacteria inoculum and duration of exposure and could not be avoided by the
155 recommended antibiotic prophylaxis (cefazolin 2 g), as it is ineffective on *S. marcescens*.

156 We would like to underline that these results were obtained through a close cooperation
157 between the hospital hygiene department, the digestive surgery ward, the hospital archive
158 department, and the microbiology laboratory. This led to the issuing of a medical device alert
159 and to the withdrawal of the T-shaped probe and showed that the use of a single-use sterile
160 sheath placed on the tip of probe during ultrasonography in laparoscopic liver resection is
161 essential in order to avoid such peroperative infections. Infection prevention and control
162 measures, including reminder courses on adequate cleaning and decontamination of the probe
163 were set up. Since then, no other case was observed.

164

165 **Conclusion**

166 We reported an outbreak of *S. marcescens* SSIs associated with peroperative
167 ultrasonography. These results led to the withdrawal of the probe, to a reinforcement of the
168 training course on the pre-cleaning step, in particular disinfection procedures vigilance, and to a

169 correction of the practices, especially by the systematic use of a single-use cover during
170 peroperative ultrasonography in laparoscopic liver resection.

171

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179

180 **Conflict of interest**

181 All authors: No reported conflicts.

182

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- 217

Tables

Table 1. Operating history and patient characteristics of *Serratia marcescens* surgical site infections, 2018, teaching hospital of Caen, Normandy, France.

Epidemic patients	Age, Sex	Medical history	Ultrasonography operation	Operation duration in hours	NNIS index ^a	Operation date	Day delay (D), prelevement type ^b	Antibiotic treatment Surgical revision	Outcome
EP1	59, male	intrahepatic cholangiocarcinoma	hepatectomy	4	2	05 April 2018	D0, biliary prosthesis	Cefepime Gentamicin	recovered
EP2	59, male	intrahepatic adenocarcinoma	hepatectomy	3	0	16 May 2018	D4, blood	Cefepime	recovered
EP3	33, female	hepatic hydatid cysts	hepatectomy	6	1	28 June 2018	D11, Redon drain	Cefepime	recovered
EP4	66, male	intrahepatic cholangiocarcinoma	hepatectomy	6	1	29 June 2018	D8, pleural fluid	Ceftazidime Surgical revision	recovered
EP5	83, female	intrahepatic adenocarcinoma	hepatic resection radiofrequency	3	0	01 August 2018	D5, blood	Cefepime Gentamicin	recovered
EP6	60, male	intrahepatic adenocarcinoma	hepatic resection radiofrequency	5	1	02 August 2018	D2, blood	Ceftazidime-avibactam Gentamicin Surgical revision	recovered
EP7	64, male	intrahepatic adenocarcinoma	hepatic resection radiofrequency	2	0	02 August 2018	D3, hepatic abscess	Surgical revision	recovered
EP8	77, male	intrahepatic adenocarcinoma	hepatic resection radiofrequency	5	1	03 August 2018	D4, Redon drain	Cefepime Imipenem	recovered

^a National Nosocomial Infections Surveillance (NNIS) index was calculated for each patient according to guidelines.⁹

^b First *S. marcescens* isolation.

Abbreviation: EP, Epidemic patient.