Wastewater drainage system as an occult reservoir in a protracted clonal outbreak due to metallo-β-lactamase-producing *Klebsiella oxytoca*

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Abstract

We describe the epidemiology of a protracted nosocomial clonal outbreak due to multidrug-resistant IMP-8 producing *Klebsiella oxytoca* (MDRKO) that was finally eradicated by removing an environmental reservoir. The outbreak occurred in the ICU of a Spanish hospital from March 2009 to November 2011 and evolved over four waves. Forty-two patients were affected. First basic (active surveillance, contact precautions and reinforcement of surface cleaning) and later additional control measures (nurse cohorting and establishment of a minimum patient/nurse ratio) were implemented. Screening of ICU staff was repeatedly negative. Initial environmental cultures, including dry surfaces, were also negative. The above measures temporarily controlled cross-transmission but failed to eradicate the epidemic MDRKO strain that reappeared two weeks after the last colonized patients in waves 2 and 3 had been discharged. Therefore, an occult environmental reservoir was suspected. Samples from the drainpipes and traps of a sink were positive; removal of the sink reduced the rate number but did not stop new cases that clustered in a cubicle whose horizontal drainage system was connected with the eliminated sink. The elimination of the horizontal drainage system finally eradicated the outbreak. In conclusion, damp environmental reservoirs (mainly sink drains, traps and the horizontal drainage system) could explain why standard cross-transmission control measures failed to control the outbreak; such reservoirs should be considered even when environmental cultures of surfaces are negative.

Keywords: Carbapenemase, environmental reservoir, IMP-8, *Klebsiella oxytoca*, outbreak
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Introduction

Outbreaks caused by multidrug-resistant (MDR) *Klebsiella* spp. are a growing worldwide problem. Such outbreaks occur more frequently (but not exclusively) in intensive care units (ICUs) [1], have been associated with significant mortality [2] and are usually clonal [3–6], although different clones or species sharing epidemic plasmids have also been described [7,8]. Most importantly, the implementation of recommended infection control measures [9] is often not enough to fully control the outbreaks, so that many evolve over long periods of time or even spread to other healthcare centre [6,10]. Specifically, the medical literature has not drawn enough attention to the potential importance of environmental reservoirs during complex outbreaks, because they have been considered less important than the reservoir formed by colonized patients [5,7].

We report a prolonged clonal outbreak of nosocomial infection due to a multidrug-resistant strain of *Klebsiella oxytoca* (MDRKO), which was previously characterized as the first IMP-8-producing *Enterobacteriaceae* in Spain [11], with the aim of describing its epidemiological features and the control measures implemented; we emphasize our finding that identifying and isolating an environmental reservoir was key for the eradication of this outbreak.

Materials and Methods

We followed the recommendations of the ORION statement for reporting outbreaks [12].

Setting

The study was conducted in La Merced Hospital, a 240-bed community public centre in Osuna, Seville (Spain). The hospital has an eight-bed medical and surgical ICU (with three additional beds in an adjoining room to be occupied if necessary), which receives ~350 admissions annually. The structure of the ICU is shown in Fig. 1. The water supply and wastewater removal system comprised 11 sinks (labelled S1 to S11 in Fig. 1); each sink drained into a wastepipe (W), labelled according to the number of the sink, except for: S6 and S7, which shared the same drainpipe and, together with S5, drained into W5; S8 and S9, which drained into W7; and S10 and S11, which drained into W8.

Patients

The investigation included all 42 patients colonized or infected by the epidemic strain of MDRKO over the outbreak, which evolved over four waves (Fig. 2). The index case was detected in March 2009 and the last case in November 2011. Patients with MDRKO were considered to be infected if presenting with signs of active infection considered to have been caused by MDRKO, according to CDC criteria [13], and colonized otherwise.

Patients, environmental and healthcare staff studies, and actions taken

Before the onset of the outbreak, all patients with >1 month stay in the ICU underwent active screening by rectal and pharyngeal swabbing for colonization with multidrug-resistant Gram negative bacteria. As described below, an active screening protocol including weekly and discharge rectal and pharyngeal swabbing (or tracheal aspirate if under mechanical ventilation) was started when the outbreak was detected.

During the outbreak, six main environmental investigations were carried out (Fig. 2 and Table 1). The earlier ones focused on dry surfaces, while later investigations tended to concentrate on surfaces surrounding the patients and damp environments. The methodology used is described in detail below.

The control measures undertaken during the outbreak are described in the Results section (the environmental measures are summarised in Table 1). An additional routine intervention carried out once a year, the Prevention and Control of *Legionella* Infection Protocol (PCLIP), which was non-specifically implemented because of this outbreak, is described here because of its potential impact on the evolution of the outbreak. The PCLIP is applied once a year to treat water supply networks of all Spanish hospitals. In our centre, this is performed by hyperchlorination of the main water tank for 3 h with free residual chlorine (20–30 mg/L) and hyperchlorination of the terminal points (1–2 mg/L) for 2 h.

As regards healthcare workers, ICU staff were screened twice during the outbreak (Fig. 2): in wave 1, a pharyngeal swab was collected, and in wave 3, pharyngeal and rectal swabs were collected.

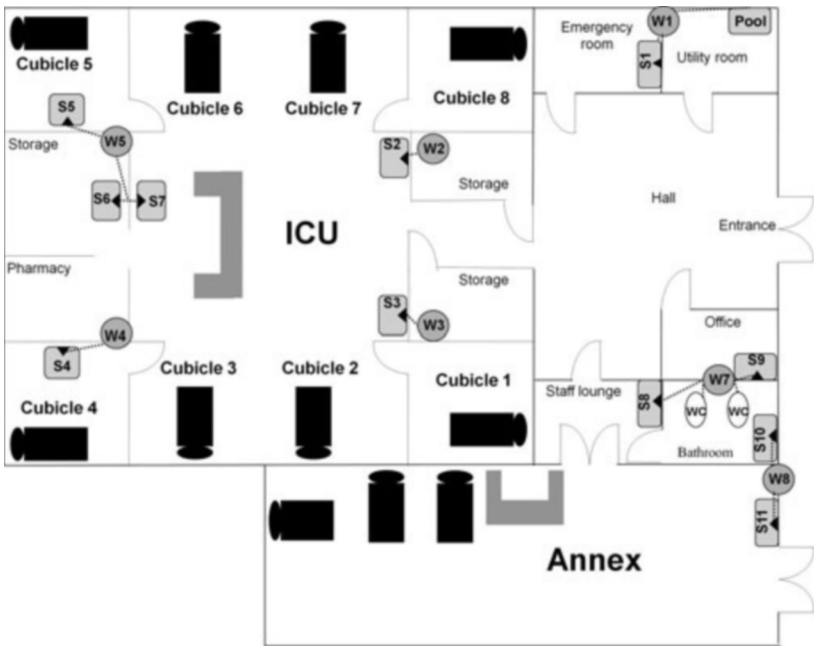

FIG. 1. Plan of the ICU. Grey square: sink. Grey circle: wastepipe. Discontinous line: drainpipe. S: sink. W: wastepipe.

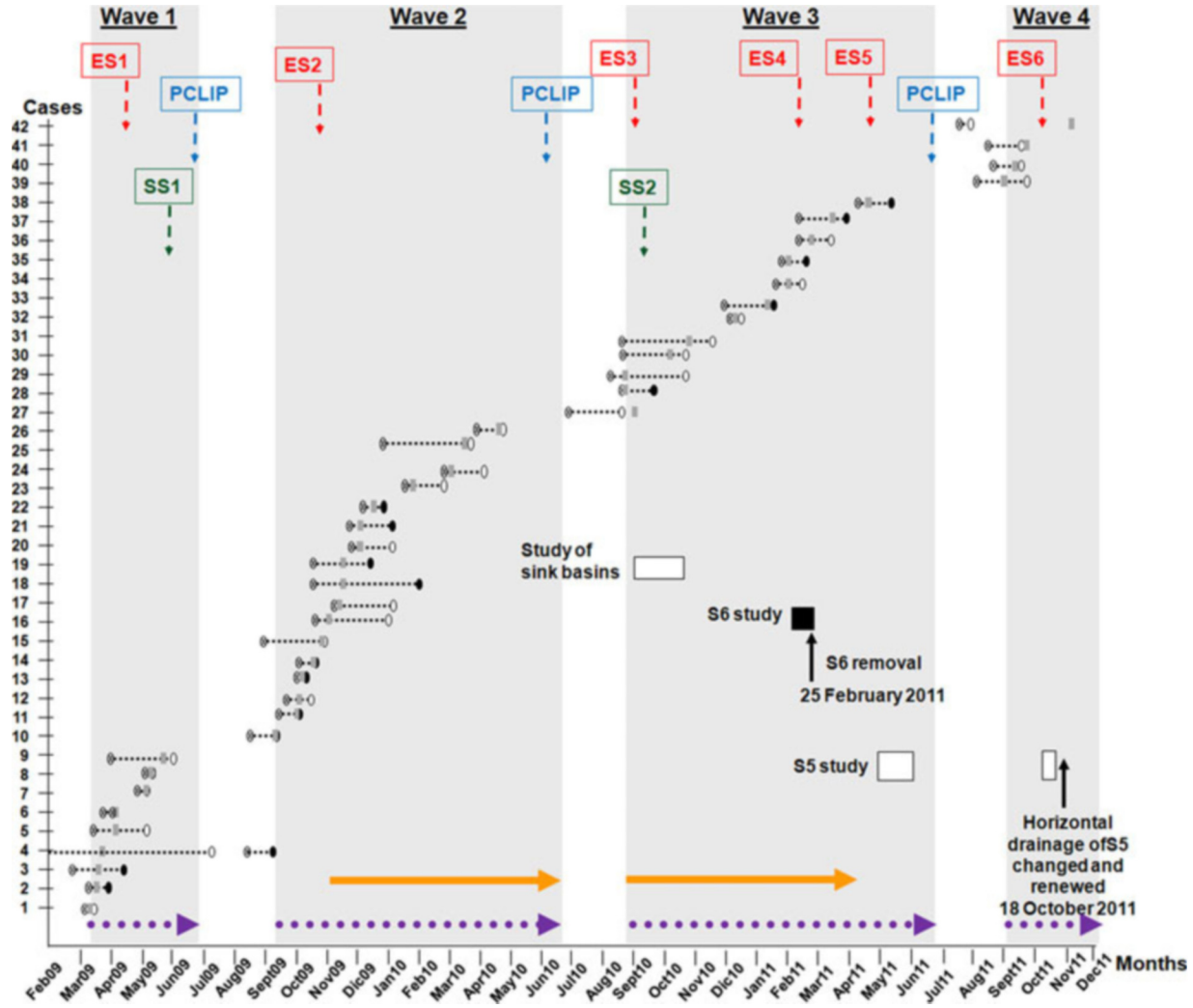

FIG. 2. Synoptic curve of patients colonized and/or infected due to multidrug-resistant *Klebsiella oxytoca* and control measures. ES (in red): environmental study. SS (in green): staff study. PCLIP (in blue): Prevention and Control of *Legionella* Infection Protocol. Discontinuous purple arrow: basic cross-transmission control measures. Continuous orange arrow: additional cross-transmission control measures. Circle with cross: admission date of patient. Open circle: discharge date of case. Black circle: date of death of case patient. Grey square: date of first isolation of MDRKO. Discontinous line: ICU stay. Black square: positive environmental culture. White square: negative environmental culture.

Antimicrobial therapy consumption

With the purpose of investigating the consumption of the main group of antibiotics in the ICU, they were grouped as follows: third-generation cephalosporins (ceftriaxone, cefotaxime and ceftazidime); fourth-generation cephalosporins (cefepime); fosfomycin; piperacillin/tazobactam; carbapenems (imipenem); glycopeptides (vancomycin); aminoglycosides (amikacin, tobramycin and gentamicin); tigecycline; and fluoroquinolones (ciprofloxacin and levofloxacin). Consumption was measured using defined daily doses (DDD) [14] per 100 patient-days.

Microbiological studies

All MDRKO isolates (i. e. isolates showing resistance to carbapenems) obtained from clinical, surveillance and/or environmental samples were studied. Screening samples were seeded onto MacConkey agar plates (Difco, Detroit, MI, USA) and chromID ESBL (BioMérieux, Marcy L'Etoile, France). From September 2010, environmental samples were also cultured in thioglycolate broth (Difco). Identification and susceptibility testing was carried out first with an automated system (MicroScan®; Siemens Healthcare Diagnostics, West Sacramento, CA, USA). The characterization of the first nine outbreak isolates, obtained between March and August 2009, was previously reported [11]. In summary, the isolates showed intermediate susceptibility or resistance to all β-lactams tested (the MICs of imipenem, ertapenem and meropenem were 2 mg/L, 1–2 mg/L and 0.5–1 mg/L, respectively), and resistance to ciprofloxacin, trimethoprim-sulpamethoxazole and

TABLE 1. Summary of environmental studies and actions

Environmental study	Wave month/year	Areas investigated	Result	Actions
1	1 April/2009	Medical equipment, medical ventilators, oxygen and air vents in walls, faucets and fibreoptic bronchoscope.	Negative	None
2	2 November/2009	Enteral nutrition preparations, telephones, tables, computer keyboards, sink surfaces, monitors, portable medical ventilators, electrocardiographs and portable X-ray equipment.	Negative	None
3	3 September/2010	Environment surrounding a patient, including instrument cases, tables, chairs, monitors and all sink basins.	MBLKO isolated from a urinary catheter and a stethoscope around a case.	On 10 September 2010, all stethoscopes were investigated. None showed a positive culture
4	3 February/2011	All sink drainpipes and traps	MBLKO isolated from S6 drainpipes and trap	On 25 February 2011, S6 and its drainage system were permanently removed. Drainage system of S7 was replaced
5	3 April/2011	Several surfaces of cubicle 5, the storage area where S6 had been installed, all sink traps and main wastepipes	Negative	None
6	4 October/2011	S5 (water from faucets, basin, overflow and drainage grille, drainpipes and traps)	Negative	S5 and S7 drainpipes connected to W4. Installation of shut-off valves in the drainpipe of every sink to carry out biweekly chemical cleaning with Biguanid®[a]

S5-6-7: sink numbers 5-6-7 in Figure 1. W4: wastepipe 4 in Figure 1.
[a]Although the environmental study was negative, a secondary environmental focus was suspected and it was decided to implement these measures (see text).

tobramycin; only fosfomycin, colistin and amikacin were active. The isolates were closely related by pulsed-field gel electrophoresis (PFGE), and were IMP-8 metallo-β-lactamase producers and chromosomal OXY β-lactamase hyperproducers [11]. *K. oxytoca* isolated after August 2009 was considered as belonging to the outbreak clone if it shared the same susceptibility profile; PFGE was also performed on selected isolates, including all environmental ones, for confirmation. Additionally, the susceptibility profiles of all *K. oxytoca* clinical isolates between February 2008 and February 2009 were retrospectively investigated.

Statistical analysis

Categorical variables are expressed as percentages and continuous variables as medians (interquartile range, IQR). We performed a chi-square test for trend for the incidence density of colonization/infection by the epidemic strain and for the percentage of cases detected by means of a rectal swab. The Kruskal-Wallis test was used to analyse the time between admission and acquisition of MDRKO, over the first three waves of the outbreak. Wave 4 was not included in the comparisons because of the low number of cases that occurred in this last wave. We also studied the trend in antimicrobial consumption before, during and after the outbreak. Epi info version 3.5.1 was used.

Results

Overall, 42 (6.4%) of the 660 patients admitted to the ICU during the outbreak period were colonized/infected by MDRKO (Fig. 2); of these, 14 (33.3%) developed an infection (Table 2). The crude mortality of colonized/infected patients was 44%. The outbreak evolved in four waves (Fig. 2 and Table 2): from March to May 2009 (nine cases), from September 2009 to April 2010 (17 cases), from September 2010 to April 2011 (12 cases), and from September to November 2011 (four cases). The incidence density of colonization/infection due to MDRKO decreased during successive waves from 1.91 (wave 1) to 1.24 (wave 2) and 0.82 (wave 3) cases per 100 patient-days. The percentage of cases that were detected by means of a rectal swab increased from 55.5% in wave 1 to 88.2% in wave 2 and 83.3% in wave 3 (p 0.02). The average time between admission and acquisition of MDRKO was 8 days (IQR, 6–37), 16 days (12–27) and 14 days (9–40) in waves 1, 2, and 3, respectively (p 0.22).

K. oxytoca isolates before the onset of the outbreak

The bimonthly average number of *K. oxytoca* isolated in the whole hospital during 2008 was 2.08 cases, but during January–February 2009, just before the outbreak started, eight *K. oxytoca* strains were isolated from different patients. Of these, three were isolated from ICU patients; one of them, isolated from a patient who was admitted to the ICU from 10 December 2008 to 5 February 2009, was an ESBL-producer but was fully susceptible to carbapenems. These isolates were not available for further microbiological studies.

Antimicrobial consumption

As shown in Fig. 3, the use of fluoroquinolones, fosfomycin and aminoglycosides significantly increased after the onset of

TABLE 2. **Main characteristics of the patients colonized and/or infected due to multidrug-resistant *Klebsiella oxytoca***

Case number/wave	Cubicle	Sex/Age	Type of admission	Date sample (month/day/year)	Type of sample	Pattern of acquisition	Discharge status
1/1[a]	8	M/53	Medical	03/13/09	TA	Colonization	Alive
2/1[a]	7	M/66	Medical	03/16/09	TA	Infection (VAN)	Dead
3/1[a]	5	M/75	Medical	03/18/09	TA/RS	Colonization	Dead
4/1[a]	1	M/75	Surgical	03/24/09	RS	Colonization	Dead
5/1[a]	5	M/37	Medical	04/04/09	TA	Infection (VAN)	Dead
6/1[a]	3	M/42	Medical	04/05/09	TA	Colonization	Alive
7/1[a]	3	M/45	Medical	05/04/09	TA/RS	Colonization	Alive
8/1[a]	6	M/80	Medical	05/10/09	PS	Colonization	Alive
9/1[a]	6	M/62	Medical	05/20/09	RS	Colonization	Alive
10/2	7	M/79	Surgical	09/07/09	B	Infection (bacteraemia)	Dead
11/2	8	M/47	Medical	09/29/09	TA	Colonization	Dead
12/2	7	M/63	Medical	10/02/09	TA/RS	Infection (VAN)	Alive
13/2	4	M/83	Medical	10/08/09	RS	Colonization	Dead
14/2	5	F/78	Medical	10/15/09	TA/RS	Colonization	Dead
15/2	6	F/71	Medical	10/27/09	RS	Colonization	Alive
16/2	1	F/41	Medical	11/03/09	RS	Colonization	Alive
17/2	5	M/67	Medical	11/10/09	TA/RS	Infection (VAN)	Alive
18/2	4	M/77	Medical	11/17/09	TA/RS/U	Urine tract infection	Dead
19/2	8	F/79	Surgical	11/17/09	RS	Colonization	Dead
20/2	8	F/52	Medical	12/02/09	TA/RS/B	Infection (bacteraemia)	Alive
21/2	8	F/80	Medical	12/03/09	TA/RS/B	Infection (bacteraemia)	Dead
22/2	1	F/50	Medical	12/16/09	TA/RS/B	Infection (bacteraemia)	Dead
23/2	2	F/61	Medical	01/26/10	RS	Colonization	Alive
24/2	3	M/74	Surgical	03/03/10	RS	Colonization	Alive
25/2	5	F/82	Medical	03/16/10	RS	Colonization	Dead
26/2	6	F/66	Medical	04/20/10	RS	Colonization	Alive
27/3	4	F/80	Medical	09/01/10	AS	Colonization	Alive
28/3	4	F/76	Medical	08/23/10	TA	Infection (VAN)	Dead
29/3	6	M/58	Surgical	08/27/10	RS	Colonization	Alive
30/3	1	M/75	Medical	10/06/10	RS	Colonization	Alive
31/3	2	M/79	Medical	10/26/10	RS	Colonization	Alive
32/3	1	F/59	Medical	12/09/10	RS	Colonization	Dead
33/3	5	M/68	Medical	01/12/11	TA/RS	Infection (VAN)	Dead
34/3	1	M/47	Medical	02/01/11	RS	Colonization	Alive
35/3	2	F/62	Medical	02/01/11	TA/RS	Infection (VAN)	Dead
36/3	7	M/70	Medical	02/22/11	RS	Colonization	Alive
37/3	5	M/57	Medical	03/15/11	RS	Colonization	Dead
38/3	5	M/76	Medical	04/19/11	RS	Colonization	Alive
39/4	5	F/76	Medical	09/03/11	Ascites	Infection (peritonitis)	Alive
40/4	3	F/69	Medical	09/20/11	TA	Colonization	Alive
41/4	7	F/63	Medical	09/23/11	TA/RS	Colonization	Alive
42/4	NA	M/68	Surgical	11/04/11	B	Infection (bacteraemia)	Alive

M, man; F, female; TA, tracheal aspirate; RS, rectal swab; PS, pharyngeal swab; B, blood culture; U, urine culture; AS, axilla swab; VAN, ventilator-associated pneumonia; NA, not applicable.
[a]Previously published data [11].

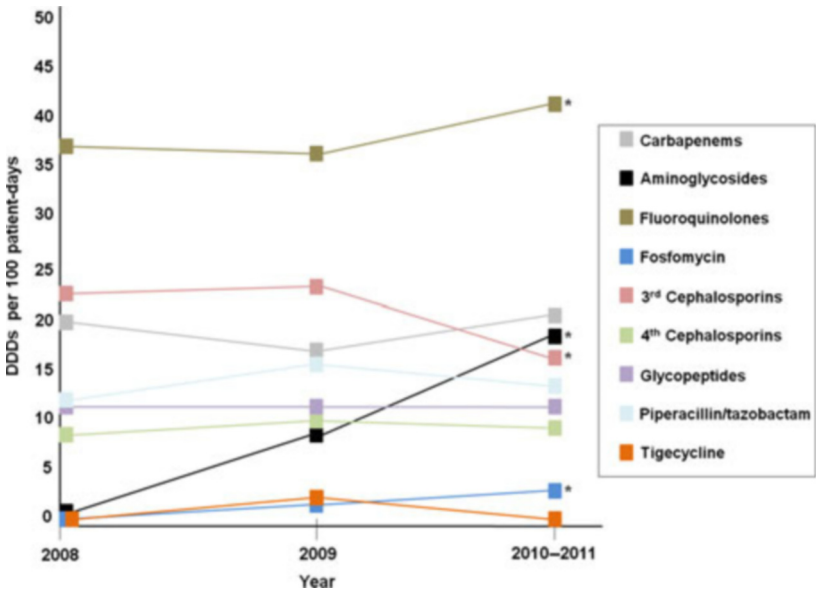

FIG. 3. Consumption of antimicrobial before, during and after the outbreak in the intensive care unit, Hospital La Merced, Osuna, Seville, Spain. DDD: defined daily dose. *p <0.05. Aminoglycosides: amikacin, tobramycin and gentamycin. Carbapenems: imipenem. Fluoroquinolones: ciprofloxacin and levofloxacin. Third-generation cephalosporins: ceftriaxone, cefotaxime and ceftazidime. Fourth-generation cephalosporins: cefepime. Glycopeptides: vancomycin.

the outbreak (the latter two were used to treat some patients infected with MDRKO). In contrast, the use of third-generation cephalosporins decreased. During the outbreak there was a non-significant reduction in the consumption of carbapenems and a non-significant increase in the consumption of piperacillin-tazobatam and tigecycline.

Description of the outbreak and control measures

Wave 1. On 19 March 2009, two MDRKO strains were isolated from the clinical samples of two patients admitted to adjacent ICU cubicles (7 and 8). Immediately, an infection control task force was formed. The basic control measures undertaken, which were repeated during later waves (Fig. 2), included: (i) active screening of all patients admitted to the ICU; (ii) contact precautions in individual cubicles for colonized/infected patients; (iii) reinforcement of standard cross-transmission control measures; (iv) periodic educational sessions; and (v) implementation of twice daily thorough cleaning. All surfaces except medical devices were cleaned with bleach (1:10 dilution of 5.25% sodium hypochlorite). Medical devices were cleaned with Biguanid® (first generation quaternary ammonium) at 1.6%. These disinfectants were tested *in vitro* at the used concentrations and showed inhibition of MDRKO growth (data not shown). The first nine isolates were submitted to a reference laboratory (Hospital Universitario Virgen Macarena, Seville) and the clonal nature of the outbreak was shown [11]. All screening samples taken from staff (SS1) and the environment (ES1) were negative (Fig. 2). The PCLIP was carried out in the hospital on 5 June. In July 2009, the last MDRKO-colonized patient still remaining in the ICU in this wave was transferred to another hospital; as no new cases had been detected by then, the outbreak was thought to have been eradicated.

Wave 2. In late August 2009, the last affected patient of wave 1 was readmitted to the ICU. Although contact precautions were implemented from admission, MDRKO was isolated from the blood culture of another patient in early September, and cross-transmission from the previous patients was suspected. All basic measures were immediately reactivated; however, new cases emerged. In November 2009, expert external advisors from the Hospital Universitario Virgen Macarena recommended 'additional measures' that were implemented, including: nurse cohorting; establishment of minimum nurse/patient and auxiliary/patient ratios of 1:2 and 1:2.5, respectively; review of the clean/dirty circuit; and reviewing the use of broad spectrum antibiotics. A second environmental study (ES2) was performed, with negative results. In April 2010, the last case in this wave was detected. The ICU and the adjoining room were left empty, walls were painted, medications removed and fomites (medication vials, gloves and containers) eliminated. On 4 June 2010, the annual PCLIP was carried out in the hospital. The outbreak was again considered to be over.

Wave 3. In late August 2010, after 4 months with no cases, MDRKO was again isolated from the clinical sample of a patient who had been admitted for only 4 days. It was noticed that a patient, who was then in the Internal Medicine ward, had previously spent a lengthy period in the ICU; this patient was screened and detected as colonized, and thus was considered the probable index case for the third wave. All basic and additional measures were again implemented. ICU staff were screened again (SS2) and all cultures were negative. During the third environmental study (ES3), a urinary catheter removed from a colonized patient and a stethoscope used with that patient yielded MDRKO. Because in February 2011 the outbreak was still out of control, a fourth environmental study (ES4), which included sinks drainpipes and traps, was carried out. Only samples from S6 were positive, showing countless colonies of MDRKO that were cultured from every pipe, trap and drainage grille sample taken; samples from the faucet or overflow grille were negative. Samples from the pipe connecting S6 and S7 were also positive. On 25 February 2011, S6 and its drain system were permanently removed and the drain system of S7 was replaced. However, another two patients admitted to adjacent cubicle 5 acquired MDRKO in March and April 2011 (18 and 53 days after the environmental intervention). A fifth environmental study (ES5) was carried out, including surfaces in cubicle 5, the adjacent storage area where S6 used to be and all the sink traps and main wastepipes of the unit (Table 1). The 18 samples taken were negative. No new cases emerged in the following months. On 17 June 2011, the annual PCLIP was performed and the outbreak was once again considered eradicated.

Wave 4. On 3 September 2011, MDRKO was again isolated from the clinical sample of a patient admitted to cubicle 5. All patients admitted were screened again; two further colonized cases were detected. A sixth environmental study (ES6) was undertaken, involving 11 samples taken from S5 (faucets, surfaces, overflow hole, drainage grilles, drainpipes and trap); all were negative for MDRKO. In spite of that and because of the high suspicion of a hidden reservoir, in October 2011 the infection-control task force decided to isolate W5, which S5 and S7 still drained into. Thus, the complete horizontal drainage system of S5 and S7 was replaced and connected up to W4. Shut-off valves were also installed to each sink drainage system. Since then, a disinfection of the drainage system is performed twice a week using Biguanid® at 1.6% for 30 min

(through closing the valves), followed by opening the valves and running hot water (70°C) for 5 min. On 4 November 2011, MDRKO was isolated from blood cultures in a patient admitted to the surgical ward. This patient and the index case for wave 4 who was discharged from the ICU on 25 September 2011, shared healthcare staff in the surgical ward; therefore it was assumed that he had acquired MDRKO during his stay in the surgical ward. Three and 6 months after the end of the outbreak, unannounced transversal screening studies of both patients and the environment were carried out, and were negative. Screening of patients with a >1 month ICU stay was resumed. No new strains of *K. oxytoca* have been detected in the hospital, as of April 2013.

Concerning the isolates not included in the earlier report [11], their susceptibility profiles were identical to the previous ones. bla_{IMP-8} was detected by PCR in all 17 isolates from wave 2, and they all showed an identical PFGE profile to the epidemic strain from the first wave. Selected isolates from waves 3 and 4 and all the environmental samples were studied for the presence of bla_{IMP-8} and molecular relatedness by PFGE profile. Every strain studied carried bla_{IMP-8} and they showed the same PFGE profile as previous isolates.

Discussion

We described one of the most prolonged nosocomial outbreaks due to carbapenemase-producing *Klebsiella* spp., which was eradicated, that to our knowledge has been described to date [1,3–8,15–18]. Since the outbreak was due to a clonal strain in a small unit that rarely admits patients from other centres, it represented an excellent model for evaluating the complex evolving nature of its epidemiology. Our results show that control measures aimed at preventing cross-transmission were partially effective but were unable to definitively eradicate the outbreak strain; this, together with the epidemiological data, strongly suggests the key role of an environmental reservoir, at least during the later waves of the outbreak.

The outbreak strain was previously characterized as the first IMP-8-producing *Enterobacteriaceae* in Spain [11]. We do not know how the carbapenemase gene entered the hospital. Because the number of non-MDR *K. oxytoca* isolates dramatically increased just before the outbreak, it may be hypothesized that the epidemic clone was spreading before acquiring the IMP-8 gene, which probably increased the chances of spreading in the context of high antibiotic use; unfortunately, *K. oxytoca* strains isolated before the outbreak were not available for typing. This hypothesis follows the theory that spread of 'susceptible' (or not MDR) clones may sometimes be the first stage of outbreaks caused by MDR bacteria if such clones eventually acquire MDR genes [19]. We also hypothesize that the gene codifying for the IMP-8 was introduced into the ICU by a colonized patient.

Colonized patients are the most important reservoir and patient-to-patient cross-transmission is considered to be the main mechanism of spread in outbreaks of nosocomial infection caused by multidrug-resistant *Enterobacteriaceae* [9]. However, it is recognized that standard infection control measures are frequently not enough to eradicate outbreaks [4,6,20]. In our case, basic control measures failed and additional measures including nurse cohorting (which was effective in other similar outbreaks [4,6]) had to be implemented. Nevertheless, the epidemic strain was not eradicated and the last waves occurred long after the last colonized patients from previous waves had been discharged. This previously observed phenomenon [3,20] may raise the suspicion of an unrecognized human or environmental reservoir that enabled the outbreak strain to survive in spite of the preventive measures to stop cross-transmission. Nevertheless, staff and patient surveillance studies did not identify any human long-term carrier.

Identifying potential environmental reservoirs has often been neglected, so that few authors to date have reported environmental studies when describing earlier outbreaks [1,3,4,6]. There are no standardized recommendations about when, where and how environmental sampling should be performed. Many of the environmental studies reported have in fact usually been carried out on dry surfaces [6]. MDR-resistant *Klebsiella pneumoniae* has been recovered from beds and various medical devices [1,6] and even from contaminated roll boards [16]. *K. oxytoca* has also been recovered from ventilator surface cultures [21]. We also found the MDRKO outbreak strain on medical instruments in the vicinity of an affected patient. However, all isolates taken from dry surfaces could merely reflect breaks in standard control measures. A stable reservoir could also be established in a moist environment where suitable conditions might favour the formation of microbial biofilms [20–24]. We found an environmental reservoir of this kind in the trap and pipes of S6 during wave 3 (Figs 1 and 2). Because of the results of environmental cultures and the association of the cases during that wave with cubicle 5, we think that a change in the epidemiology of the outbreak occurred, evolving from a predominantly patient-related reservoir during the first wave (although we cannot discard water drain system involvement) to an environmental one.

Importantly, contaminated sinks and drainage systems are becoming more frequently identified as relevant reservoirs of MDR Gram-negative bacteria, including *Acinetobacter baumannii*

[23], *Pseudomonas aeruginosa* [24] and recently also ESBL-producing *K. oxytoca* [20]. Contaminated water (i.e. used for staff hand washing, hygiene of patients or washing devices) is drained through the sinks; biofilm-forming bacteria may form stable reservoirs in the waste pipes and even in the semi-horizontal drain pipes if they are not inclined enough or are partially blocked. Water splashing from the faucets creates an aerosol effect from the sink's drain, which may later contaminate the basin and surrounding surfaces [23,24]. In our outbreak, removing the S6 and S7 drainage system failed to completely eradicate the outbreak strain; even though we had eliminated the S6 reservoir, S5 remained connected to the S6 pipe and to W5 (Fig. 1), and new cases occurred in cubicle 5. It was only when the horizontal drainage system to S5 was removed and S5 and S7 were connected to W4 that the outbreak was finally brought under control. We think that S5 was also a reservoir that could not be detected.

Finally, an unintended action may have played an important role in the epidemiology of the outbreak. In the first three waves, the emergence of new cases was stopped, coinciding with the application of the PCLIP, only to remerge a few months later (Fig. 2). This would suggest that the PCLIP may have had a significant but not decisive impact on the contaminated drainage systems, perhaps because it was unable to completely eradicate the bacterial biofilms. Chemical disinfection of the drainage system has been previously reported to have had little impact on the bacterial burden in attempts to solve similar problems [20,24].

In conclusion, the epidemiology of outbreaks due to carbapenemase-producing *Enterobacteriaceae* may be complex and evolving; even when the main reservoirs are formed by colonized patients, and cross-transmission is the principal means of their spread, alternative reservoirs should be suspected if strictly applied traditional control measures are not efficacious. In these circumstances, a wet environmental reservoir (mainly the drain, trap and horizontal drainage system of a sink) should be considered. Finally, initiatives standardizing environmental investigations in healthcare settings should be encouraged.

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Transparency Declaration

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