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Persisting transmission of carbapenemase-producing *Klebsiella pneumoniae* due to an environmental reservoir in a university hospital, France, 2012 to 2014

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In France, the proportion of episodes of carbapenemase-producing *Enterobacteriaceae* (CPE) with no recent stay or hospitalisation abroad is increasing. In this study, we investigate epidemiological links between apparently unrelated cases of OXA-48-producing *Klebsiella pneumoniae* (Kp OXA-48) colonisation or infection. We genotyped detected organisms by repetitive sequence-based PCR, and used a dynamic registry of cases and contacts to cross-reference patients' hospital stays. Between 1 November 2012 and 28 February 2014, 23 Kp OXA-48 cases were detected in a university hospital in Montpellier, of which 15 were involved in three outbreaks: outbreaks I and II occurred in November 2012 and outbreak III in October 2013. Molecular comparison of bacterial strains revealed clonal identity between cases involved in outbreaks II and III and four single cases. Cross-referencing of hospital stays revealed that these single cases and the index case of outbreak III had occupied the same room. Active case search among former occupants of that room found an additional Kp OXA-48 carrier. A clonal strain was isolated from the sink of that room. The epidemiological link between the contaminated room and outbreak II remained undetected. This study is a reminder that environmental reservoirs should be considered as a source of CPE transmission.

Introduction

Since the 2000s, rates of carbapenemase-producing *Enterobacteriaceae* (CPE) have increased worldwide [1] and become endemic in several European countries [2]. *Enterobacteriaceae* cause various infections (urinary tract, digestive or respiratory infections) and the presence of carbapenemase increases mortality rates [3,4].

In France, where CPE are still considered emergent and mostly imported from Mediterranean countries, no link with a foreign country (hospitalisation or travel abroad of the index case) was reported for half (819/1,625) of the events (defined as one or more epidemiologically related CPE cases) notified by infection control teams and/or laboratories between January 2004 and March 2015 [5]. The most frequently found CPE in France is OXA-48-producing *Klebsiella pneumoniae* (Kp OXA-48) and in 2014, 656 episodes were notified [5].

In our healthcare facility, a teaching hospital in southern France, three outbreaks of Kp OXA-48 infections and colonisations occurred in November 2012 and October 2013 and several single cases occurred in 2013. While one of these single cases was imported from North Africa, the remaining could not be linked to an epidemiological source, raising the question of unidentified bacterial reservoirs either within our hospital or circulating in the community. The aim of this study was to investigate epidemiological links between Kp OXA-48-positive patients, with no evident epidemiological source of transmission and seemingly unrelated, that occurred in our facility between 1 November 2012 and 28 February 2014.

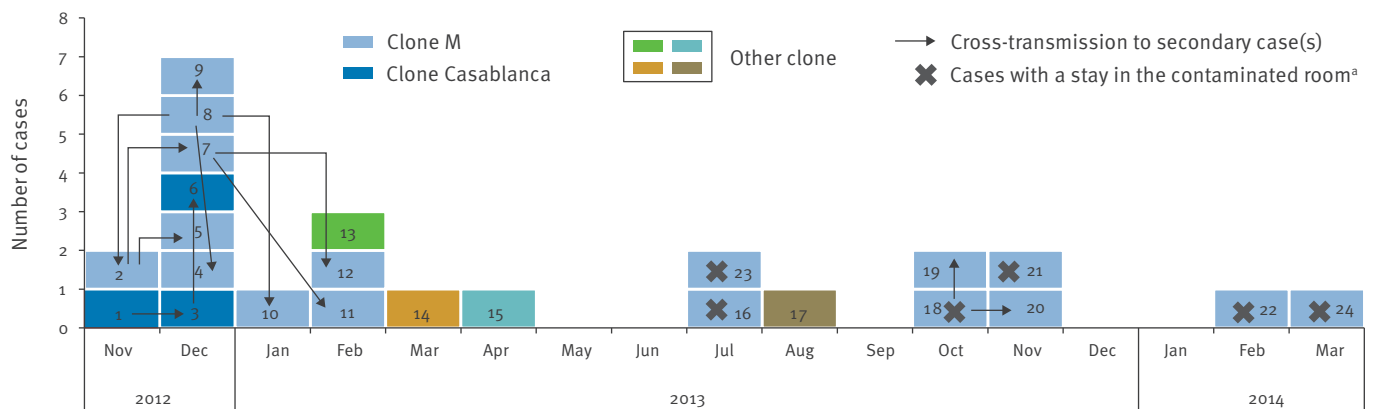
Methods

Setting

The study was conducted in the University Hospital of Montpellier, a 2,634-bed tertiary care teaching hospital, organised in five distinct hospital sites. It has seven intensive care units (ICU), including a 12-bed neurosurgical ICU. The Infection Control (IC) team comprises 1.6 full-time doctors, seven nurses and an attached IC

FIGURE 1

Epidemic curve of OXA-48-producing *Klebsiella pneumoniae*, University Hospital of Montpellier, France, 1 November 2012–28 February 2014 (n = 24)



One square represents one case. Cases are numbered by order of inclusion in the investigation. Each colour represents a different clone of OXA-48-producing *K. pneumoniae*.

^a One intensive care unit room where OXA-48-producing *K. pneumoniae* was isolated from environmental samples.

laboratory. Clinical wards are regularly visited to evaluate healthcare professionals' compliance with standard precautions, hand cleaning and hospital hygiene. In January 2014, a dynamic registry of CPE cases and contacts (an ongoing Excel file) [6] was set up to facilitate case management, contact tracing and alert upon readmission of cases or uncleared contacts (incompletely screened contacts, see study definitions). All CPE cases and contacts diagnosed in our hospital from October 2012 onwards were retrospectively registered, and all incident cases and contacts thereafter.

Multidrug-resistant organism surveillance policy (implemented in 2006)

According to French recommendations, all patients with more than 48 hours continuous stay in the ICU undergo active screening (weekly nasal and rectal swabs) for multidrug-resistant organisms (MDRO). In other units, screening is performed on patients presenting risk factors (history of previous MDRO carriage, transfer from a long-term care facility, chronic wounds and/or indwelling medical device). Since 2013, in response to national recommendations [7], patients transferred from a foreign hospital or with a history of hospitalisation abroad in the previous 12 months have been screened for MDRO and CPE upon admission. A daily automatic report from the microbiology laboratory informs the IC team of prevalent MDRO-positive clinical or screening samples.

Hospital hygiene and environmental control policy

Nursing auxiliaries trained in procedures written by the IC team carry out the cleaning of patients' rooms. The protocols include daily disinfection of sinks with bleach solution at a concentration of 0.5% of available chlorine, with at least one hour of contact.

Environmental surveillance is performed by the IC laboratory and involves regular screening of sinks on high-risk wards and sinks on any ward with a history of contamination. Each ICU sink is screened twice a year by sampling tap water and tap and trap surfaces. A more comprehensive sampling of dry and damp surfaces is performed during outbreaks for the detection of potential reservoirs.

Study definitions

Cases of *Kp* OXA-48 were defined as patients (infected or colonised) identified in our facility between 1 November 2012 and 28 February 2014, with a *Kp* OXA-48-positive culture from any site during their hospitalisation. An outbreak was defined as at least two cases linked by an epidemiological chain of transmission: an index case followed by one or more hospital-acquired secondary cases, with indistinguishable bacterial strains according to molecular biology. A sporadic case was defined as a single case, or the index case of a cluster, that couldn't be linked to an epidemiological source.

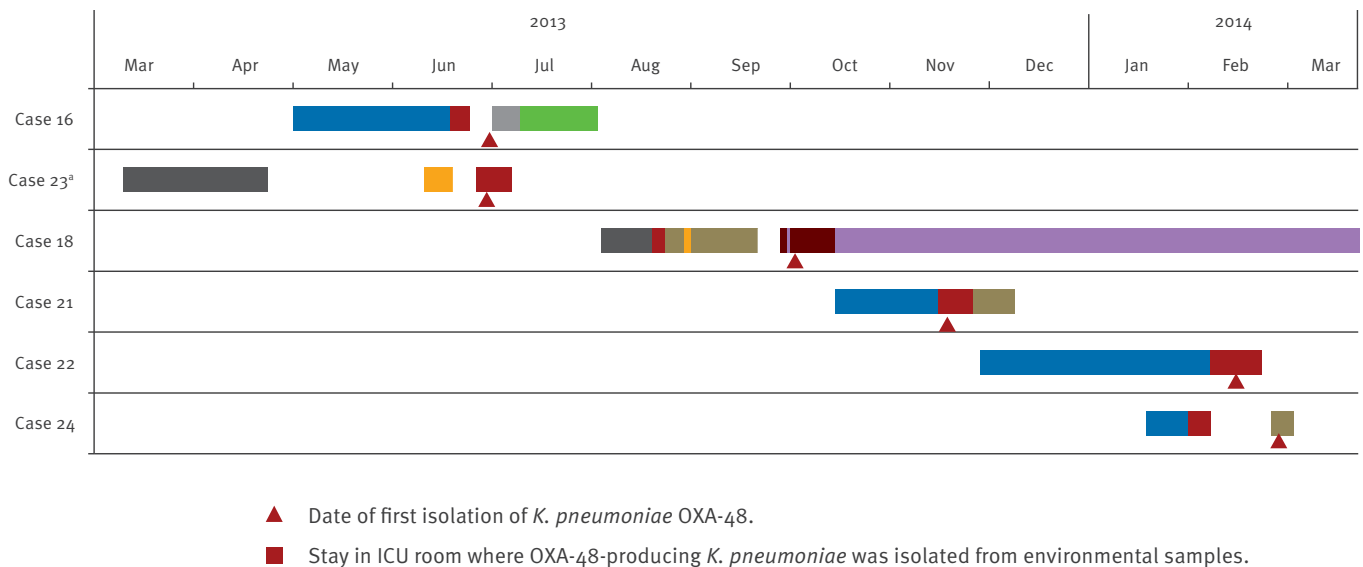
Contacts were the patients cared for by the same healthcare team as a case. Their screening (repeated weekly rectal or stool swabbing) was followed up until three negative results.

Microbiological studies

Clinical strains were isolated during routine practice of medical microbiology according to clinical laboratory policy. Briefly, detection of CPE was performed using a combination of different media to screen for OXA-48 and other CPE (chromID CARBA SMART, bioMérieux, France). The resistance profile was interpreted according to the recommendations of the Antibiogram Committee of the French Microbiology Society (CA-SFM).

FIGURE 2

Synoptic curve of sporadic cases with the same clone of OXA-48-producing *Klebsiella pneumoniae*, University Hospital of Montpellier, France, March 2013–March 2014 (n=6)



Each colour represents a different ward.

^a Patient identified retrospectively because of an initially mistaken identity.

When suspected from selective media and resistance profile, the presence of the carbapenemase gene was confirmed by the regional reference laboratory (Nîmes University Hospital) using the Check-MDR CT102 microarray (Check-Points, the Netherlands). Bacterial strains were compared by in-house repetitive sequence-based PCR (rep-PCR) [8].

Environmental samples (surfaces and sinks) were taken with sterile, cotton-tipped swabs. After a specific search for *Enterobacteriaceae* on selective medium (Mac Conkey Agar), matrix-assisted laser desorption/ionisation (MALDI) time-of-flight (TOF) mass spectrometry was performed for identification.

Results

Characteristics of cases

Between 1 November 2012 and 28 February 2014, 24 Kp OXA-48-positive patients were identified in the University Hospital of Montpellier. Their epidemiological characteristics are shown in Table 1.

Two outbreaks occurred in November 2012 (outbreaks I and II) and one in October 2013 (outbreak III); they involved three, nine and three cases, among which 12 were hospital-acquired secondary cases (Figure 1). Cases are numbered by order of discovery in the course of the investigation. Case 23 was included later than the discovery date, in spite of an early positive Kp OXA-48 finding, because of a mistaken identity at the regional laboratory.

Outbreaks I and II happened simultaneously (indeed, the second one was revealed through contact tracing of the first), and involved two distinct bacterial clones in rep-PCR (data not shown). Outbreak I occurred from an index case (case 1) transferred from a Moroccan hospital (clone Casablanca) and generated two secondary cases (cases 3 and 6); in outbreak II, clone M was found in nine patients (cases 2, 4, 5, 7 to 12) and stemmed from an index case (probably case 8) with no known source of contamination.

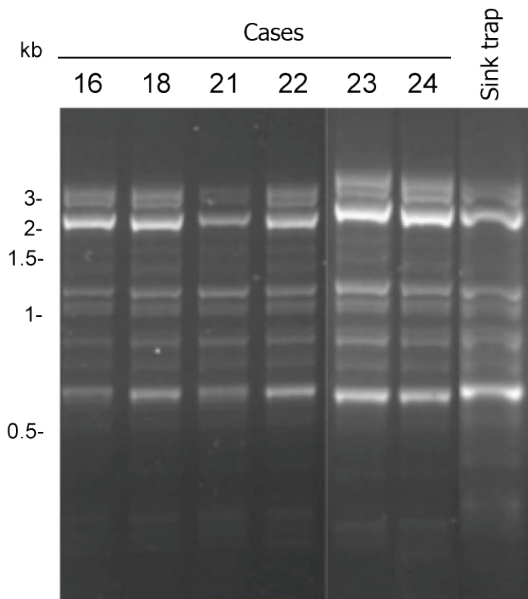
Seven sporadic cases of Kp OXA-48 (cases 13 to 18 and 21) were identified in 2013: six remained single cases and one (case 18) was the source of outbreak III (two secondary cases, cases 19 and 20). Among these sporadic cases, only one (case 13) had a history of health-care in a foreign country. For the six others, no contact with a known CPE carrier was found and three had previously negative MDRO screenings. A recent hospitalisation or residency in a long-term healthcare facility was found for three of the six cases and no significant history was found for the three other cases.

In February 2014, a new case (case 22) was diagnosed in the neurosurgical ICU, also seemingly unrelated to any source of contamination. At the same time, we were informed by the regional laboratory that a misidentified case from July 2013 was to be considered (case 23). By February 2014, a total of nine sporadic cases were under investigation.

A comparison of all the bacterial strains was performed by rep-PCR in February 2014 (data not shown).

FIGURE 3

In-house rep-PCR profiles obtained for clinical (n=6) and environmental (n=1) *Klebsiella pneumoniae* strains belonging to clone M, France, 1 November 2012–28 February 2014



The origin of the strain is indicated at the top of the gel. The nomenclature of strains follows the numbering of the cases. Sizes in kb corresponded to 1 kb ladder.

It showed that the three cases involved in outbreak III also belonged to clone M identified in outbreak II. More surprisingly, it also revealed that four of the single cases (cases 16, 21, 22 and 23) shared that same clone M profile. Overall, clone M was found in 16 cases: nine from outbreak II, three from outbreak III and four single cases. The clone Casablanca was not identified in other than the three cases of outbreak I; four different clones were diagnosed in the remaining four single cases (cases 13, 14, 15 and 17).

Epidemiological investigation

Using the registry of CPE cases and contacts, cross referencing of the cases' hospital stays highlighted that four of the sporadic cases (cases 16, 18, 21 and 23) had occupied the same room in the neurosurgical ICU before detection of their Kp OXA-48, following one another at intervals of two to 84 days between June and December 2013 (Figure 2). All four were colonised with the epidemic clone M. The patient present in that room at the time of investigation, in February 2014, also turned out to be colonised by Kp OXA-48 (case 22). Retrospective case search among patients admitted to this room in the three months before the investigation detected one additional case (case 24).

In all, six cases with clone M had been hospitalised in this ICU room between June 2013 and February 2014. Five of these cases were men and their median age was 43 years (range: 23–51); their underlying conditions were severe traumatic head or spine injury (n=4) or haemorrhagic cerebrovascular events (n=2). Kp OXA-48 was isolated from a rectal swab in four of these cases and from tracheal aspiration in the other two. All six patients were considered as colonised and none received antibiotic treatment for a clinical infection involving the epidemic bacterial strain. No other epidemiological link was found between these six cases, and no contact was found between them and the cases of outbreak II.

Environmental investigation

Thirty-nine swabs were taken on different dry surfaces and five on damp surfaces of the involved ICU room on 21 and 25 February 2014 (while the room was occupied by case 22). The room had a single bed and a hand washing sink. Two samples from the siphon and the tap aerator of the water outlet yielded Kp OXA-48. This bacterium was not detected on the dry surfaces of the room, the nursing station or adjacent bedrooms. Comparison of environmental strains with the six patients who had occupied the room showed identical pulsed-field gel electrophoresis (PFGE) profiles (Figure 3). Thorough cleaning and surface disinfection were performed and new sink trap and tap were installed; extensive environmental sampling performed in March 2014, after the intervention (total: 55 samples), did not find Kp OXA-48. No additional sporadic case was identified after implementation of the environmental measures.

Discussion

We report here the persistent transmission of a single Kp OXA-48 clone and provide arguments in favour of a role of moist environments in the transmission of CPE. Water and water outlets are well-reported reservoirs for nosocomial transmission of *Pseudomonas aeruginosa* [9,10], and the risk of acquiring multidrug-resistant (MDR) bacteria from prior room occupants in ICU has been demonstrated for MDR *Acinetobacter baumannii*, *P. aeruginosa* [11] and organisms such as methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci [12]. The role of an environmental source in the transmission of extended spectrum beta-lactamase-producing (ESBL) *Enterobacteriaceae* [11,13] has been underestimated in spite of outbreak reports supporting the evidence [14,15]. As for CPE outbreaks, patient-to-patient cross-transmission is the privileged hypothesis, supported by numerous reports of negative environmental investigations [16–20]. However, a few outbreaks with environmental transmission of CPE have been described in Australia, Spain and Norway [21–23]; these protracted outbreaks (20 to 30 months duration) occurred in ICUs between 2007 and 2012. A recent meta-analysis has established that the risk of MDRO acquisition from prior occupants is as important for Gram-negative as for Gram-positive organisms [24].

TABLE

Epidemiological characteristics of OXA-48-producing *Klebsiella pneumoniae* cases diagnosed in Montpellier University Hospital, France, 1 November 2012–28 February 2014 (n = 24)

Characteristics	Cases
Sex ratio male/female	1.7
Age in years, median (min–max)	62 (23–85)
Link with a foreign country in the previous 12 months (hospitalisation abroad)	2
Cases involved in outbreaks	15
Index cases	3
Secondary cases	12
Single cases	9
Length of stay in days in a hospital unit ^a , median (min–max)	11 (1–104)
Clinical infections	4

^a Only stays in the Montpellier University Hospital are considered.

Transmission of microorganisms from a contaminated sink trap to patients is commonly attributed to splashing [25], either directly on the patient or onto health-care professionals' hands. It has been reported that hospital room design is a key element in environmental contamination by MDRO [15,25]. It has also been suggested that rates of environmental contamination are higher for EBSL *K. pneumoniae* than EBSL *Escherichia coli* [26,27].

In our study, despite the daily chlorination process, the epidemic clone was identified from the siphon of the sink in room occupied by a Kp OXA-48-colonised patient. The direction of the contamination can be questioned (the positive patient could have contaminated the sink) and it was not possible to determine the origin of the environmental strain. However, there are indirect arguments in favour of a sink-to-patient contamination route. Firstly, this patient had prior negative MDRO screenings before their stay in this room and was otherwise unrelated to the other cases with the same clone. The same was true for the case retrospectively detected among prior occupants of the room. Secondly, no further case acquired in our hospital has been identified after the corrective works on the incriminated water outlet.

We were not able to establish the transmission link between the patients sharing the ICU room and outbreak II (involving the same bacterial clone). Other cases may have gone undetected among prior occupants of the room, as we did not call them all back for extensive screening. Furthermore, a study carried out from February 2011 to February 2013 in our region found a clonal diversity among Kp OXA-48 strains identified in the region [28], and the circulation of a community strain with the same PCR profile seems unlikely.

Hence, the hypothesis of a missing link in the nosocomial transmission chain remains unresolved.

In our study, molecular epidemiology proved a useful complement to classical investigation methods. Indeed, a transmission link between the cases was not straightforward, as they were not grouped in time and space when their first CPE-positive culture was known. The molecular findings prompted a thorough investigation of these apparently unrelated sporadic cases and revealed an unsuspected environmental reservoir. Even if cross-transmission remains the privileged hypothesis when investigating a CPE outbreak, as rates of Kp-OXA 48 cases increase in our hospitals, our study reminds us to consider environmental reservoirs as a source of CPE transmission.

Conflict of interest

None declared

Authors' contributions

Conception and design: A. Lotthé/S. Parer/B. Clarivet; acquisition of data: B. Clarivet/D. Grau/E. Jumas-Bilak/H. Jean-Pierre, analysis and interpretation: B. Clarivet/E. Jumas-Bilak/A. Pantel, redaction: B. Clarivet / A. Lotthé, final approval of the version to be published: all the authors.

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