ORIGINAL ARTICLE

VIM-*Klebsiella oxytoca* outbreak in a Neonatal Intensive Care Unit. This time it wasn't the drain

R. HERRUZO[1], G. RUIZ[2], S. GALLEGO[3], J. DIEZ[3], A. SARRIA[2], F. OMEÑACA[4]
[1] Department of Preventive Medicine and Public Health and Microbiology, School of Medicine, Autonomous University of Madrid, Spain;[2] Microbiology Service, Universitary Hospital "La Paz", Madrid, Spain; [3] Preventive Medicine Service, Universitary Hospital "La Paz", Madrid, Spain; [4] Neonatology Service, Children Hospital.Universitary Hospital "La Paz", Madrid, Spain

Keywords

VIM-*Klebsiella oxytoca* • NICU • Sink (drain)

Summary

Objective. *We describe an outbreak of VIM-carbapenemase Klebsiella oxytoca (VIM-Kox) in a NICU*
Materials and methods. *Prospective Epidemiological Surveillance:*
a) Systematically (weekly screening cultures) or on admission, if the patient had a history of previous colonization by VIM-Kox.
b) Clinical cultures, done if infection was suspected.
c) Other possible microorganism sources were investigated: their mothers (rectal microbiota), milk packages and preparation apparata in the lactodietary section, echocardiagram transductors, cribs, the sinks (faucets and drains), washing bowls, etc.
Molecular typing was performed using the DiversiLab (bioMérieux) system on all VIM-Kox isolated from environment or patients (one by neonate).
Results. *We identified 20 VIM-Kox cases, the most only presented colonization, but 4 showed infection. Three of the ten sinks (drains) in our NICU, were positive for VIM-Kox. Another four drains harbored P.aeruginosa, S. maltophilia and/or Enterobacter sp. Nevertheless the VIM-Kox bacteria in the sinks (drains) were not the same as those in the patients, who showed three different strains.*
Conclusions. *A VIM-Kox colonization or infection outbreak in a NICU is described. Rather than environment, not even drains, the source of the outbreak was other patients. The outbreak was relatively brief, as a result of the rapidness with which appropriate measures were taken and followed.*

Introduction

The frequency of carbapenemase-producing microorganism isolation in tertiary hospitals has been rising since 2007 [1], particularly *Enterobacteriaceae* [2-6]. Molecular biology techniques have detected antibiotic resistance genes like carbapenemase Ambler types A, B and D [7]. The Class B or metallo-betalactamases can be divided into three different types: the IMP, VIM and NDM.
When a new patient is colonized or infected by microorganisms with carbepenemases, it is necessary to determine whether this is due to microorganisms from other patients or from reservoirs that are difficult to clean during the inter-patient room cleaning/disinfection [8-12].
These reservoirs have sometimes been seen with hydrophilic bacteria like *P. aeruginosa*, *B. cepaciae*, or *Klebsiella oxytoca* with carbepenemase associated with sink contamination [13-18]. The drains of these sinks can harbor biofilms, which, in addition to hampering disinfection, can facilitate microorganism survival, gene interchange among microorganisms, and later, their dispersion as aerosols that can contaminate patients or healthcare workers when the sink is used for personal hygiene [18].
The microorganisms typing is useful to diagnose transmission and also allows us to detect if there is an outbreak or only a cluster of unrelated cases and establish monitoring systems to survey compliance with the prevention measures.
Carbepenemase-producing *K. oxytoca* were previously isolated in patients from some Spanish hospitals [1, 19-21]. Although this microorganisms can harbour plasmids with VIM-metallo-betalactamase (VIM-Kox), we had not found any reference to a previous outbreak among neonates. In this paper we report an outbreak by VIM-Kox in a NICU at a tertiary children's hospital, and describe the preventive measures taken to control further spread of this microorganism.

Materials and methods

La Paz Children's Hospital is a tertiary hospital with one NICU. Since 1985 monitoring and control of hospital infection is performed by one medical epidemiologist (part-time) and one nurse epidemiologist (dedicated full time).
Different multidrugs resistant microorganisms surveillance strategies are employed, including surveillance of clinical microbiology laboratory results, obtained during clinical care, and routine screenings to detect asymptomatic colonization.

Epidemiological surveillance for infection or colonization by these microorganisms is performed in two ways:
1. systematically, with an active surveillance methodology using weekly screening cultures taken from all children admitted to the NICU;
2. at admission, if the patient has a previous multidrugs resistant microorganisms colonization history.

A "VIM-Kox-case" is determined by the identification of *K. oxytoca* with VIM-carbepenemase in any biological sample taken from the patient (catheter tip, bronchoalveolar exudate, blood, conjunctiva, throat, rectal, etc.), regardless of the presence of symptoms. On some occasions, a patient was colonized by different *VIM-Enterobacteriaceae* genera.

Bacteria frequency was measured as a "cummulative incidence" of cases in a given time period (new cases divided by the number of children admitted during this period in our NICU, multiplied by 100).

A) Microbiological method

Surveillance studies in patients:

A.1) Clinical Samples

In neonates with any symptom of infection, urine, blood, broncho-alveolar lavages and other samples based on the most likely focus of infection were taken.

Antibiotic susceptibility was determined in clinical samples using the Wider (Fco. Soria Melguizo, Madrid, Spain) or Vitek (bioMérieux, Marcy l'Étoile, France) systems. Isolates were categorized as susceptible or resistant to any of the antibiotics tested following CLSI guidelines. Tigecycline Minimun Inhibitory Concentration (MIC) were evaluated according to the interpretative criteria of the FDA. Extended-spectrum β-Lactamase production was confirmed by E-test extended-spectrum β-Lactamase strips (bioMérieux) and carbapenem MIC were confirmed by E-test (BioMérieux). To rule out carbapenemase production, a modified Hodge test was performed on all *Enterobacteriaceae* isolates retrieved from clinical cultures having an MIC ≥ 1 mg/L to imipenem or meropenem and an MIC ≥ 0.5 mg/L to ertapenem. The inhibition tests with boronic acid and EDTA were used to screen for the production of class A and class B carbapenemases.

A.2) Surveillance Samples

Weekly samples were obtained from the neonates' pharynx, nose, feces, catheter entry points and connections, and the incubator water.

These samples were cultured in MacConkey agar supplemented with 4mg/L of cefotaxime (Tec-Laim, Madrid, Spain). Disc diffusion and a modified Hodge test were performed on all *Enterobacteriaceae* isolates to identify extended-spectrum β-Lactamase, plasmid-mediated AmpC and carbapenemase production.

We mapped the VIM-colonized (or infected) patients within the NICU. Carbapenemase genes were confirmed by PCR (Progenie Molecular, Valencia, Spain). Molecular typing was performed using the DiversiLab (bioMérieux) system on all KoVIM isolated from environment or patients (one by neonate).

B) Control measures

The bundles recommended for controlling VIM-*Enterobacteriaceae* were adapted from those described in CDC 2012 [21-24] (Tab. I).

Our Epidemiologist-Nurse evaluated implementation of these measures daily, reporting any compliance failures to the healthcare workers. The Medical-Epidemiologist reinforced these daily recommendations with the NICU supervisors for doctors and nurses.

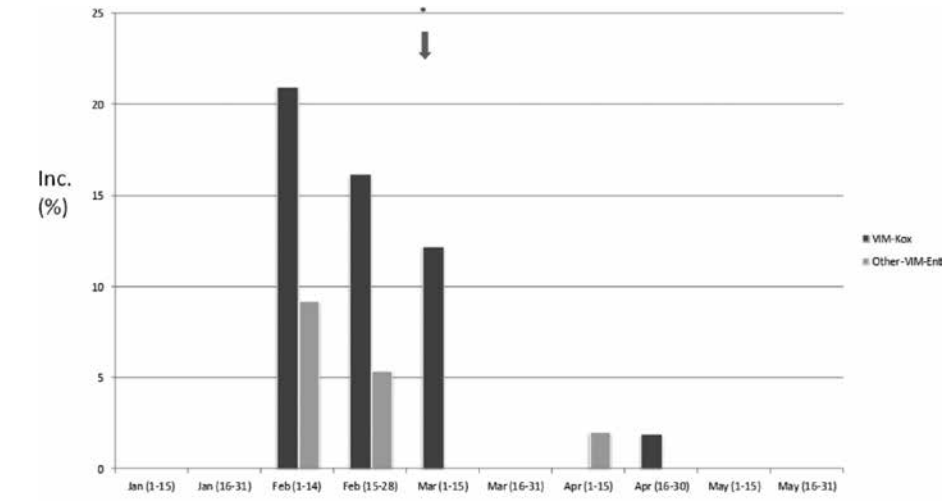
C) Statistical method

We studied the data for frequencies of children infected/colonized per week, (incident cases), plotting an epidemic curve.

Tab. I. Measures used in NICU by to control the outbreak of *VIM-K. pneumoniae*.

a) Early detection and implementation of contact precautions, emphasizing hand hygiene with alcohol solutions. The efficacy of the alcohol solution actually used in neonates was tested with VIM-microorganism recently isolated in our NICU.
b) Cohorting the VIM cases, grouping them in one specific area of the NICU.
c) Cohorting the healthcare workers, especially nurses. In the first month, physicians were also dedicated to the VIM-cases, but after that time, they also cared for other non-VIM patients.
d) Restriction of β -lactam-antibiotic use in neonates and limitation to sensitive antibiotics (according to the antibiogram) if the neonate carried VIM- microorganisms.
e) Flagging the patient's clinical history with a green-colored page stating the contact precautions, used when the child was taken out of the unit for clinical tests, etc. This same signalling page was used if the child was readmitted to our hospital.
f) Daily body washing used a 0.1%-0.5% aqueous chlorhexidine solution (0.1% in preterm < 4 weeks of life or a term < 2 weeks; in preterm > 4 weeks of life or a term > 2 weeks, chlorhexidine is used at 0.5%).
g) Restriction of number of healthcare workers from other specialties who came to visit neonates.
h) Information sessions for parents and refresher courses for NICU doctors, nurses, assistants, and specialists, held to explain the epidemiological evolution of VIM bacteria and the steps to be taken during each phase.
i) The possible environmental origins of this VIM bacteria outbreak were explored early on, and included sinks, NICU surfaces, disinfectants, eyewashes, echograph-transductors etc., that could be related with the outbreak. Milk from the Dietetary Service, instruments and milk recipients were sampled, and also the water faucets in the NICU were studied fortnightly (between February and March). The samples were taked with swabs immediately before bein immersed in Tood-Hewitt broth.
j) Other measures taken to interrupt the epidemiological evolution of the outbreak were to test the efficacy of disinfectant used on surfaces (double application of diluted quaternary ammonium and isopropyl-alcohol to the same surface) with VIM –microorganisms from our NICU, on a glass-germ-carrier, as previously described [21, 24].

Fig. 1. Cummulative incidence (%) for VIM-*K. Oxytoca* vs other VIM *Enterobacteriaceae* in NICU.



arrow = more extreme contact precautions

Results

The VIM-Kox NICU outbreak began in the first week of February 2014. The weekly microbiota control sample (performed weekly on Thursdays) identified 8 VIM-Kox cases (4 also had VIM-Serratia). There were another 3 patients in whom other VIM -*Enterobacteriaceae* were identified. The results for all the patients with multidrugs resistant microorganisms were received on the Monday of the second week of February, and all neonates with these microorganisms were placed under contact precautions, but it was not possible to establish an isolated cohort. Thursday that week, samples were again taken, and another six neonates had become VIM-Kox positive. Consequently, the first fortnight in February had a VIM-Kox cummulative incidence of 21% (Fig. 1), 40 times higher than during the two previous years.

The preventive measures mentioned in "Materials and methods" were taken.The effectivity of the hand antiseptic against the bacteria recently isolated from patients was studied and found to be very effective within 15 seconds. Moreover, the double application of diluted quaternary ammonium and isopropyl-alcohol to the same surface, destroyed, in vitro, all VIM-microorganisms studied. Consequently, it was not necessary to change these methods of antisepsis and disinfection.

Last, a cohort with VIM-neonates was established. However, it was not possible to include all the affected babies, since, due to their underlying disease not all could be moved into the cohort isolation area.

In the second fortnight of February, VIM-Kox cummulative incidence was slightly lower (16%). At this point other possible microorganism sources were investigated: their mothers (rectal microbiota), sink drains, milk packages and preparation apparata in the lactodietary section, echocardiagram transductors, cribs, the sinks (faucets and drains), washing bowls, etc. Everything, except for three of the ten sinks, were negative for VIM-Kox. Another four drains harbored *P.aeruginosa*, *S. maltophilia* and/or Enterobacter sp. (Fig. 2). Next, the susceptibility of these VIM-*Enterobacteriaceae* to different non-chlorinated desinfectants was determined. Chlorinated products were not used because they may emit gases that could irritate the neonate respiratory tract. All VIM-bacteria in this outbreak were susceptible to chlorhexidine, oxygen peroxide or alcohol, and moderately resistant to diluted quaternary ammonium. Once susceptibility was determined, the drains were disinfected by pouring 1 liter of 5% clorhexidine down the drain. This was effective in two of the three VIM-Kox-affected sinks. The third sink required, as we have done in other bacterial outbreaks, an application of steam (vapor) and after a chemical disinfectant (in this case a mixture of 3% hydrogen peroxide and lactic acid, which had given a good in vitro result). This treatment was applied in the ten affected sinks and the subsequent controls were negative in all cases for VIM-Kox as well as other multidrugs resistant microorganisms. Additionally, the number of HCW from other Services who were attending the neonates was restricted and in the third fortnight, the cumulative incidence had dropped to 12% and then 0% in the fourth fortnight after the outbreak began. There was only one new case in April with none in May or June, at which time the outbreak was considered over (Fig. 1).

As seen in Figure 2, the locations of incubators with cases were widely scattered throughout the unit, and were not particularly related with the sinks in which VIM-Kox had been isolated. Moreover, the Microbiology Laboratory confirmed via genetic analyses that the sink´s VIM-Kox were different to those of the cases and, among the 20 cases, there were three different strains. The first strain, isolated in the first fortnight of February (cases 1 to 8) remained until the first fortnight in March (case 15) affecting patients in the three "areas" (303, 304 and 308); The second strain spread during the second

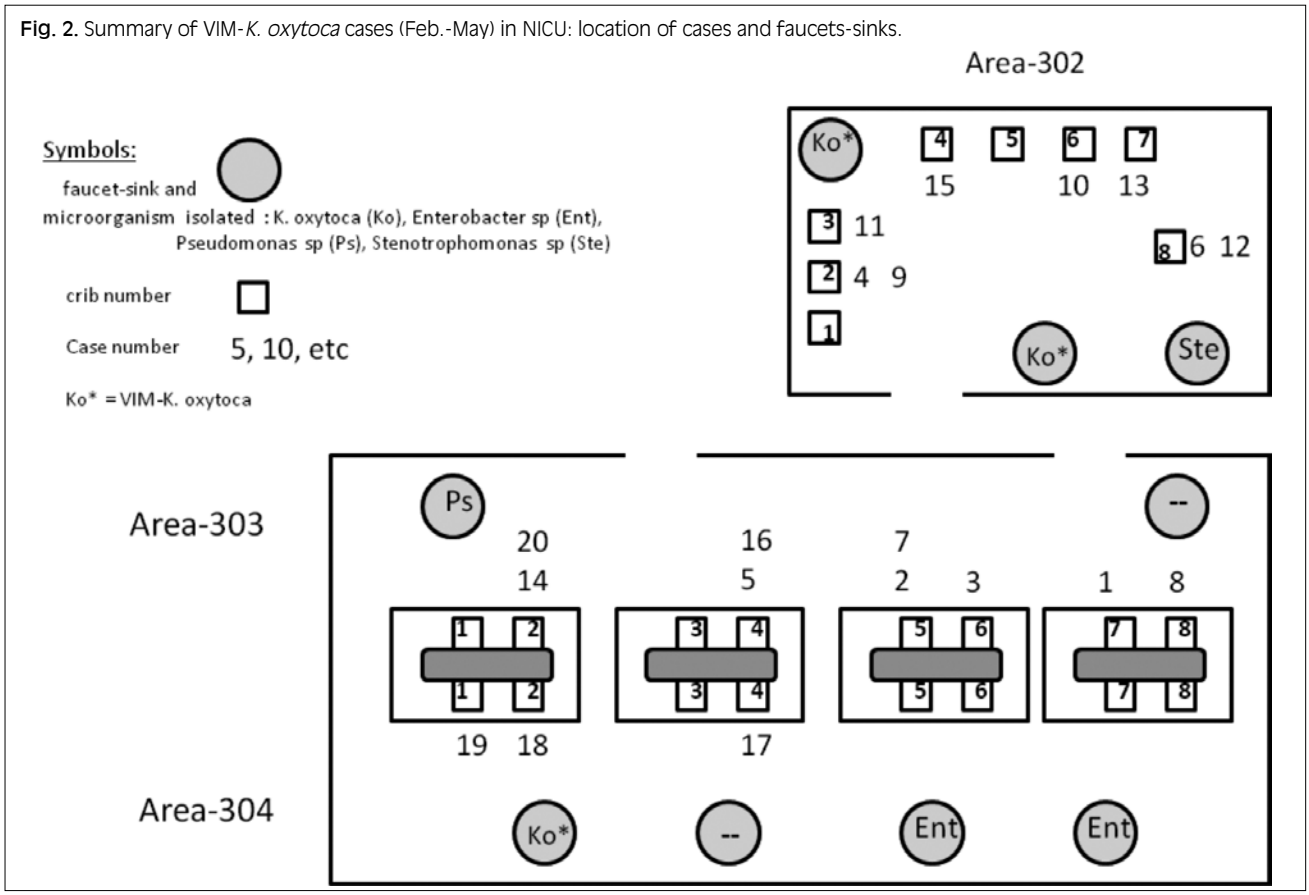


Fig. 2. Summary of VIM-*K. oxytoca* cases (Feb.-May) in NICU: location of cases and faucets-sinks.

fortnight in February (cases 9 to 14) and first fortnight in March (cases 17 to 19), also with cases in the three NICU areas, and, in this fortnight in March, a third strain was identified, but only in 303 area (case 16).

During the outbreak, three children were admitted to the hospital and found to be already VIM-Kox positive, colonized in another hospital, and their data are not considered in the analysis of the outbreak.

The third of these children was admitted in the first fortnight in April, and the last incident case to be detected in this outbreak was detected in the second fortnight of the month (case 20); he was probably, although we do not have the genetic study, infected by the outside case since:

1) it had been nearly a month since the last case;

2) they were near each other in the same room;

3) in the first two weekly controls in which they were both sampled, case 20 was still negative, only becoming positive at the end of the second fortnight in April.

In all, the NICU had 20 cases of VIM-Kox (4 of them had VIM-Kox and VIM-Seratia and 6 with other VIM-Enterobacteriaceae). Of the VIM-Kox cases most were only colonizations but 4 also had infection (3 pneumonias and one conjunctivitis, Table II). These bacteria were susceptible to various antibiotics: Ertapenem, Meropenem, Amikacin, Colistin, Tigeciclin and quinolones. In each patient, the median of surveillance studies from admission to event was 3.

VIM-Kox was also isolated in 6 other infants admitted outside the NICU, and there were 3 patients (coming from other hospitals) that entered the NICU already colonized with VIM-Kox.

Discussion

VIM-Kox has been few isolated in our neonates by epidemiological surveillance during the two previous years [21], with an incidence between 0.1% and 0.3%. Other hospitals, or even the community [25], may be the reservoirs of these microorganisms. Several studies [13-17] have related *K. oxytoca* outbreaks with very damp environmental reservoirs, like sink drains in patients' areas or the sinks in ICU's. On this occasion, these microorganisms were found in 30% of the sink (drains) in our NICU, but the genetic analysis showed that the sink VIM-Kox´s were different from those in the neonates. Nevertheless the sinks were disinfected with heat plus chemicals and the VIM-Kox microorganisms were eliminated from that reservoir (at least temporarily).

Separation into two cohorts (with and without VIM bacteria) as well as applying contact precautions have given good results in prior outbreaks (with an OR > 5 for infected and OR > 30 for colonized patients [24]), and were enacted as soon as possible, producing a large reduction in incidence (Fig. 1) as soon as the measures were in place.

However at times it was impossible to transfer the neonates in whom VIM- bacteria had been detected in the weekly sampling to the isolation area on the day colonization was microbiologically confirmed, and this may

Tab. II. Neonates with VIM-K. oxytoca, according to colonizatior or infection by these bacteria.

Case	Rectal col.	Ot. col. sites	Infection	Antibiotics adm.	Outcome
1	Yes	No	No	No	Favourable
2	Yes	Yes (pharinx)	BN	CTX;AM;Va;Cla	Favourable
3	Yes	Yes (pharynx)	No	No	Favourable
4	Yes	No (pharynx)	BN	CTX;G;Va;Me	Favourable
5	Yes	Yes	No	CTX; Va;Amp	Favourable
6	Yes	No	Conj.	CTX; Va	Favourable
7	Yes	Yes (pharynx)	No	No	Favourable
8	Yes	No	No	CTX; Va	Favourable
9	Yes	No	No	No	Favourable
10	Yes	No	No	Cla;G;Amp	Favourable
11	Yes	No	No	Amp;CTX; G	Favourable
12	Yes	No	No	No	Favourable
13	Yes	No	No	Amp;Va;CTX	Exitus*
14	Yes	No	No	No	Favourable
15	Yes	No	No	Va;AM	Favourable
16	Yes	Yes (pharynx)	BN	Amp;G;Cla;Me	Favourable
17	Yes	No	No	No	Favourable
18	Yes	No	No	No	Favourable
19	Yes	No	No	No	Favourable
20	Yes	Yes (pharynx)	No	CTX; Va;AM	Favourable

BN = bronchopneumonia; Conj = Conjunctivitis; col = colonization; Ot.Col. = other colonization; adm = administrated. CTX = Cefotaxime; AM = Amikacin; Va = Vancomycin; Cla = Clarithromycin; G = Gentamicin; Amp = Ampicillin; Me = Meropenem.
*exitus no related with any infection

have placed the neonates in their immediate surroundings at risk, although the neonate with possible multidrug resistant microorganisms had been placed under contact restrictions from the time of their first suspected diagnosis. From these facts, it follows that the main cause of VIM-Kox´s transmission was that the lack of adherence to contact precautions measures by some health care workers.
In any hospital outbreak it is necessary to evaluate the effectiveness of the existing antiseptic and disinfection protocols against the outbreak organisms. The already recommended alcohol solution for hands, and surface desinfectants, were effective against the patients' VIM-Kox strains.
In addition to all of the above, these measures, which suppose an organizational challenge within the NICU, must be accompanied by refresher hygiene training for all healthcare workers, as well as regular updates on the evolution of the outbreak so as to increase or maintain the existing measures and evaluate if there have been any failures so that these failures can be corrected as soon as possible.

Study limitations

1) Antibiotic effect has not been evaluated directly in neonate fecal microbiota, since microorganism diagnosis was qualitative, not quantative, and only detected the presence or absence of a given bacteria, but not its quantity in a given sample weight. Quantification would have made it possible to use this variable as an indicator of recent contamination or of susceptibility to VIM-bacteria multiplication (in competition with the other intestinal microbiota).
2) Microbiota studies more frequently than once a week have not been possible, even at the peak of the outbreak (differently from previous outbreaks), possibly delaying the establishment of precautionary measures like contact control and cohort grouping for children with recently acquired VIM-bacteria by a few days, and slightly increasing the possibility of multidrugs resistant microorganisms transmission to other neonates.
3) Compliance with control measures (hand samples, observation at established times, etc.) has not been objectively evaluated, and evaluation has only been qualitative by observation and speaking with healthcare workers who did not complete all the steps for controlling these multidrug resistant microorganisms. What is more, epidemiological surveillance was only done during the morning shift, occasionally in the afternoon, and never in the evening shift.

Conclusions

- A VIM-Kox colonization/infection outbreak in a NICU is described.
- The outbreak was relatively brief, as a result of the rapidness with which appropriate measures were taken and followed (at least during the time in which compliance was directly observed).
- The already recommended alcohol solution for hands and surface desinfectants were effective against the patients' VIM-Kox strains.
- The source of the VIM-Kox microorganisms was other patients, but not the mothers or environment (milk, milk preparation material, NICU apparata, etc.), or even the drains in the NICU (which, however, were reservoirs for other VIM-Kox strains).

Acknowledgements

We thank NICU Secretary and health care workers.
The authors declare that they have no competing interests.
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

RH and FO conceived and designed the research. GR, AS and RH performed the microbiological analysis. SG and FO, collected the epidemiological data. RH and JD, performed the statistical analysis. RH and JD, wrote the manuscript. All authors revised and approved the final manuscript. Revision of the text by a native English speaker (C. Warren).

References

[1] Tato M, Coque TM, Ruiz-Garbajosa P, et al. *Complex clonal and plasmid epidemiology in the first outbreak of infection involving Enterobacteriaceace NIM-1 metallo-B-Lactamase in Spain. Toward endemicity?* Clin Infect Dis 2007;45:1171-8.

[2] Paño-Pardo JR, Ruiz-Carrascoso G, Navarro-San Francisco C et al. *Infections caused by OXA-48-producing Klebsiella pneumo-*

niae in a tertiary hospital in Spain in the setting of a prolonged, hospital-wide outbreak. J Antimicrob Chemother 2013;68:89-96.

[3] Walsh TR, Weeks J, Livermore DM, et al. *Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study*. Lancet Infect Dis 2011;11:355-62.

[4] Zaoutis TE, Goyal M, Chu JH, et al. *Risk factors for and outcomes of bloodstream infection caused by extended spectrum B-lactamase-producing Escherichia coli and Klebsiella species in children*. Pediatrics 2005;115:942-9.

[5] Canton R, Akova M, Carmeli Y, et al. *Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe*. Clin Microbiol Infect 2012;18:413-31.

[6] Gupta N, Limbago BM, JB Patel, et al. *Carbapenem-resistant Enterobacteriaceae: Epidemiology and prevention*. Clin Infect Dis 2011;53:60-7.

[7] Nordmann P, Carrer A. *Les carbepenemases des enterobacteries*. Arch Pediatr 2010;17:S154-S162.

[8] Otter JA, Yezli S, Salkeld JAG et al. *Evidence that contaminated surfaces contribute to the transmission of hospital pathogens and an overview of strategies to address contaminated surfaces in hospital settings*. Am J Infect Control 2013;41:S6-S11.

[9] Huang SS, Datta R, Platt R. *Risk of acquiring antibiotic-resistant bacteria from prior room occupants*. Arch Intern Med 2006;166:1945-51.

[10] Weber DJ, Anderson D, Rutala WA. *The role of the surface environment in healthcare associated infections*. Curr Opin Infect Dis 2013;26:338-44.

[11] Han JH, Sullivan N, Leas BF, et al. *Cleaning hospital room surfaces to prevent health care associated infections*. Ann Intern Med 2015;163:598-607.

[12] Lerner A, Adler A, Abu-Hanna J et al. *Environmental contamination by carbapenem-resistant Enterobacteriaceae*. J Clin Microb 2013;51:177-81.

[13] Lowe C, Willey B, O'Shaughnessy A, et al. *Outbreak of extended-spectrum β-Lactamase–producing Klebsiella oxytoca infections associated with contaminated handwashing sinks*. Emerg Infect Dis 2012;18:1242-7.

[14] Vergara-Lopez S, Domınguez MC, Conejo MC, et al. *Wastewater drainage system as an occult reservoir in a protracted clonal outbreak due to metallo-b-lactamase-producing Klebsiella oxytoca*. Clin Microbiol Infect 2013;19:490-8.

[15] Scotta C, Juan C, Cabot G, et al. *Environmental microbiota represents a natural reservoir for dissemination of clinically relevant metallo-B-lactamases*. Antimicrob Agents Chemother 2011;55:5376-9.

[16] Kotsanas D, Wijesooriya WRPLI, Korman TM, et al. *"Down the drain": carbapemem-resistant bacteria in intensive care unit patients and handwashing sinks*. MJA 2013;18:267-9.

[17] Ferrnti G, Marchesi I, Favale M, et al. *Aetiology, source and prevention of waterborne healthcare-associated infections: a review*. J Clin Microbiol 2014;63:1247-59.

[18] Leitner E, Zarfel G, Luxner J, et al. *Contaminated handwashing sinks as the source of a clonal outbreak of KPC-2 producing Klebsiella oxytoca on a Hematology Ward*. Antimic Ag Chemother 2015;59:714-6.

[19] Miró E, Segura C, Navarro F, et al. *Spread of plasmid containing the bla-VIM-1 and bla-CTX-M genes and the qnr determinant in Enterobacter cloacae, Klebsiella pneumonia and Klebsiella oxytoca isolated*. J Antimicrob Chemother 2010;65:661-5.

[20] Peña I, Picazo JJ, Rodríguez-Avial C, Rodríguez-Avial I. *Carbepenemase-producing Enterobacteriaceae in a tertiary hospital in Madrid, Spain: high percentage of colistin resistance among VIM-1-producing Klebsiella pneumoniae ST11 isolates*. Int J Antimicrob Ag 2014;43:460-4.

[21] Herruzo R, Ruiz G, Burgos C, et al. *If you are looking for, you can find endemic bla-VIM gene microorganisms, in children´s Hospitals*. Global Adv Res J Microbiol 2016;5:42-9.

[22] CDC. Guidance for Control of carbapenem-resistant *Enterobacteriaceae* (CRE). 2012.

[23] Herruzo R, Vizcaino MJ, Herruzo I. *In vitro-in vivo sequence studies as a method of selecting the most efficacious alcohol-based solution for hygienic hand disinfection.* Clin Microbiol Infect 2010;16:518-23.

[24] Herruzo R, Ruiz G, Rubio M, et al. *Controlling an outbreak of Pseudomonas aeruginosa in a Neonatal Intensive Care Unit: multivariate analysis of risk factors through a case-case-control study*. J Neonatal Biol 2014;3:163.

[25] Tsakris A, Poulou A, Markou F, et al. *Dissemitation of clinical isolates of Klebsiella oxytoca harboring CMY-31, VIM-1 and a new OXY-2-type variant in the community*. Antimic Ag Chemother 2011;55:3164-8.

■ Received on November 28, 2016. Accepted on November 3, 2017.

■ Correspondence: Rafael Herruzo, Department of Preventive Medicine and Public Health and Microbiology. School of Medicine. Universidad Autónoma de Madrid C/Arzobispo Morcillo, 4. 28029 Madrid (Spain). E-mail: rafael.herruzo@uam.es