Clinical Case Report

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Bla-OXA48 gene microorganisms outbreak, in a tertiary Children's Hospital, Over 3 years (2012–2014)

Case Report

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Abstract

Rationale: Carbapenem-resistant Enterobacteriaceae are an emerging problem in children. Nosocomial spread remains the principal risk factor for acquisition of these microorganisms.

Patients concerns: We describe an outbreak of *Klebsiella pneumoniae* OXA48 (KOXA48) in a tertiary children's hospital during the years 2012 to 2014, as well as the preventive measures put in place in colonized and infected cases.

Diagnoses: We studied, "in vitro," the KOXA48 susceptibility to antiseptics and surface disinfectants. Moreover, an epidemiological surveillance of infection or colonization by these microorganisms, with molecular typing of the KOXA48, was performed, and carbapenemase genes were confirmed by polymerase chain reaction (PCR).

Interventions: The bundles recommended (early detection, cohorting of children and health care workers [HCW], contact precautions, etc.) to control the KOXA48 outbreak were taken from those described in the centers for disease control (CDC) 2012 guide, and adapted according to our experience in controlling other outbreaks.

Outcomes: All the KOXA48 microorganisms isolated from children belonged to the same strain (ST11) and were susceptible to alcohol solutions but not the surface disinfectant previously employed in our hospital (tensoactive). We reinforced the surface disinfection using a double application (tensoactive + alcohol). The outbreak of KOXA48 begun in 2012 (16 cases in neonatal intensive care unit [NICU] and 1 in pediatric intensive care unit [PICU]) ended before the end of the same year and was not transmitted to new patients in 2013 to 2014, despite readmission of some colonized cases, in intensive care units (ICUs) and other units, of our children hospital.

Lessons: Infected children are the tip of the iceberg (3/17) of KOXA48 prevalence making it necessary to identify the cases colonized by these bacteria. At the beginning of the outbreak, the susceptibility of the epidemic strain to antiseptics and surface disinfectants should be studied. Moreover, the measures taken (cohorts, contact precautions, etc.) must be thorough in both colonized and infected cases, immediately, after microbiological diagnosis.

Abbreviations: ATCC = American type culture collection, Bla = betalactamases, CDC = centers for disease control, CFU = colony forming units, CLSI = Clinical and Laboratory Standards Institute, EDTA = ethylenediaminetetraacetic acid, ESBL = extended-spectrum β-lactamases, FDA = Food and Drug Administration (USA), HCW = health care workers, ICU = intensive care unit, IQR = inter-quartil-range, KOXA48 = *Klebsiella pneumoniae* OXA48, KPC = *Klebsiella pneumoniae* carbepenemase, NDM = New Delhi metallobetalactamases, NICU = neonatal intensive care unit, PCR = polymerase chain reaction, PICU = pediatric intensive care unit, ST = sequence-type, VIM = Verona -metallobetalactamases.

Keywords: Bla-OXA48, control, outbreak, tertiary Children's-Hospital

Editor: Eric Lau.

Disclosures: The authors have no funding to disclose.

The authors report no conflicts of interest.

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Medicine (2017) 96:40(e7665)

Received: 2 June 2016 / Received in final form: 3 July 2017 / Accepted: 6 July 2017

http://dx.doi.org/10.1097/MD.0000000000007665

1. Introduction

The frequency of carbapenemase-producing microorganism isolation in tertiary hospitals has been rising since 2007,[1] particularly *Klebsiella pneumoniae* in urinary tract infections, surgical sites, septicemia, and ventilation-associated pneumonia.[2–6] Molecular biology techniques have detected antibiotic resistance genes such as Ambler types A, B, and D carbapenemases.[7,8] The class A carbapenemase in *K pneumoniae* (KPC) was the first identified type to be found in the USA. Class B, or metallobetalactamases (which contain zinc in their active site), are the Verona -metallobetalactamases (VIM) and New Delhi metallobetalactamases (NDM) types described in Verona and New Delhi, respectively. Class D includes OXA48, isolated in Turkey, Israel, and other Mediterranean countries.[3,9,10]

They all produce beta-lactamases and some also present porin modifications, another mechanism that produces antibiotic resistance. They may be eliminated in the intestine through competition with flora without these bacterial resistance

mechanisms, including bacteriocins and nutritional competition,[11] if antibiotic pressure is reduced over the long term (often more than 1 year).[9] Moreover, some of these microorganisms have diverse virulence factors that facilitate the host colonization and infection, mainly in *K pneumoniae* species.

It is essential to reduce transmission either by patient-to-patient contact and from contact or with the hands of health care workers (HCW). Microorganism typing is useful to diagnose transmission pathways and also allows us to detect if there is an "outbreak" or only a cluster of unrelated cases.

Besides designing appropriate measures for each microorganism we need to establish monitoring systems to survey compliance with these measures, as these often require behavioral changes that are poorly followed by HCW (especially physicians), or are soon forgotten if not reinforced regularly.[12–14]

Finally, control measures should also affect the cleaning and disinfection of surfaces[15–19] in the patients' environment such as sinks, faucets, doorknobs, etc., which may be reservoirs for these carbapenemase-producing microorganisms.

Carbapenem-resistant *Enterobacteriaceae* are an emerging problem in children.[20] Normally, these bacteria are found in infected children in oncology or intensive care units. Nosocomial spread remains the principal risk factor for acquisition of these microorganisms in developed countries. Moreover, microorganisms with bla-OXA48 are more frequently endemic than epidemic, and these microorganisms have very rarely been reported in children.

In this paper, we describe an outbreak of a strain of *K pneumoniae* OXA48 carbapenemase-producing (KOXA48) in a tertiary children's hospital during the years 2012 to 2014, as well as the preventive measures put in place for infected and colonized children.

2. Material and methods

2.1. "In vitro" studies

The microorganisms were control (American type culture collection [ATCC]) or clinical /epidemiological samples. The ATCC was *K pneumoniae* 13883. The rest of microorganisms were collected from clinical samples or the fecal microbiota of La Paz University Hospital patients: 3 strains of *K pneumoniae* with OXA48-carbepenemase, isolated from the first 3 cases diagnosed in children (all these strains belonged to the same sequence-type: ST-11).[19,21]

We used 4 alcohol-based solutions as antiseptics: Daromix-solución (0.2% biguanidine, 2-propanol, and ethanol) Lab José Collado, Spain. Sterillium (0.2% mecetronium, 1–2-propanol) Lab Bode-Chemie GmbH, Germany. NDP-derm (N-duopropenide, ethanol); Lab Vesismin, Spain. Alcoaloe (70% ethanol, 0.2% chlorhexidine, 0.1% benzalkonium chloride, 1% phenoxyethanol); Lab A Govantes, Spain. This product is normally used in our hospital. The disinfectants studied were, Sanitbio (1.6 g% benzyl chloride C12, C18 alkyldimethylammonium, 1.5 g% chlorine-didecyl dimethylammonium chloride, and 1.6 g% of benzyl-C12, C14 alkyl dimethyl, and <5 g of anionic surfactants, Lab Proder-Pharma, used diluted 200 times), and sodium hypochlorite (10% sodium hypochlorite, Lab Guinama, used diluted 100 times, that is, 1000 mg/L). Lastly, the antiseptic or disinfectant control was 70% 2-isopropyl-alcohol.

Other materials to test the activity of antiseptic and disinfectants were 2 germ-carriers, glass beads, and the biocide neutralizer. Germ-carrier-1 was lyophillized pig skin (a model of skin), Germ-carrier-2 (a model of surface) was glass cover-slides, sized 12×35 mm. Glass beads had 0.5 mm in diameter, and the neutralizer of antiseptic/disinfectant activity was a mixture of Nutrient broth with Tween-80 at 6% + 0.5% sodium bisulphite + 0.5% sodium thiosulfate.

2.1.1. Methods. The in vitro method to detect the efficacy of antiseptics (alcohol-based solutions) was similar to 1 previously described,[21] with a high correlation with in vivo tests in efficacy results, but without requiring exposition of volunteers to outbreak strain and adapted to very short times (20 or 30 seconds).

The in vitro method to detect the efficacy of surface disinfectant[19] was: we chose 12 mm $\times$ 35 mm glass cover slides as germ-carriers, to be contaminated and then disinfected, since they are easily manageable standard surfaces. They were placed horizontally on parallel glass bars, which had been disinfected previously by flame. After placing the cover slides, we poured/dripped 10 μL of a 24 hours culture media (diluted to 1/20 or undiluted) onto the center of each slide and allowed it to dry completely (1 hour). Flame-sterilized forceps were used to grasp a cover slide and to wipe it 5 times with a disinfectant impregnated cloth. Three types of cleaning cloths were also tested: new or used microfiber or single-use cotton. One milliliter of disinfectant was placed on the surface of a 3×5 cm piece of cloth and 10 seconds later the cloth was used. The disinfectant was left to act for 15 minutes and then we introduced the glass germ-carrier in a test tube with 5 mL of a disinfectant activity neutralizer and 0.5 g of sterile glass beads, vortexing it during 3 minutes at 2000 revolutions/min to elute surviving colony forming units (CFU) from the germ-carrier.

Next, 2 0.1 mL aliquots of the supernatant liquid were cultured on MacConkey agar; Difco (Lawrence) and incubated at 37 °C for 48 hours, after which we took the CFU count. In order to make counting easier, we made dilutions at 1/10 and 1/100 of a third sample of 0.1 mL of this same liquid that had been treated and counted in the same way.

The CFU for the control were also calculated similarly, but using 1 mL of sterile distilled water instead of disinfectant on the cloth. Three samples of 0.1 mL were sown on MacConkey plates: 0.1 mL directly from the control, and 2 from dilutions at 1/100 and 1/10,000 (if not, the CFU number would have been too high for proper counting).

2.2. Surveillance studies

The La Paz Children's Hospital is a tertiary hospital that offers all the pediatric specialties: general pediatrics, dialysis; oncology; infectious diseases, transplants and surgery for cardiac, digestive, orthopedic, and other pathologies. This hospital has 2 Intensive Care Units: Pediatric (PICU) and Neonatal (NICU). Since 1980 monitoring and control of hospital infection is performed by a medical epidemiologist (part-time) and a nurse epidemiologist (dedicated full time). Over the years they have described and controlled numerous hospital outbreaks caused by microorganisms such as *Serratia*, *Pseudomonas*, *Enterobacter*, etc.,[22–24] as well as endemic infections.[25]

This work is based on a minimum data-set of each patient, collected in epidemiological records, by the Preventive Medicine Service, to assist in the control of the outbreak. Then, ethical approval was not necessary.

All children are monitored from their entry in ICU, collecting in a file their risk factors of infection, etiology and consequences of it, stay, etc.

Different KOXA48 surveillance strategies have been employed, including surveillance of clinical microbiology laboratory results obtained during routine clinical care and routine screenings to detect asymptomatic colonization.

Epidemiological/microbiological surveillance of infection or colonization by these microorganisms was performed in 2 ways: systematically with an active surveillance methodology using weekly screening cultures (swabs of catheter tip, throat, rectal), taken from all children admitted to the 2 ICUs, or every 2 weeks for the patients in Oncology and Transplants.

The second surveillance method employed clinical cultures that were performed if infection was suspected or if the patient had a previous colonization history (swabs of catheter tip, conjunctiva, throat, etc. or culture of blood, broncho-alveolar exudates, urine, etc).

A "KOXA48-case" was determined by the isolation of KOXA48 in any biological sample from patients (catheter tip, broncho-alveolar exudates, blood, conjunctiva, throat, rectal, etc.), regardless of the presence of symptoms; after identification these patients were divided into "colonized-cases" (without infection) or "infected-cases" (colonization plus infection, or only infection, diagnosed from clinical samples taken due to suspicion of infection).

Frequency of KOXA48 is measured as a "percentage" of cases by month:

(a) "Incidence": New cases, divided by the number of children admitted that month in each ICU, multiplied by 100 (i.e., "cumulative incidence")
(b) "Prevalence" (total of cases in a month, either, new or diagnosed earlier cases) in each month, divided by the number of children admitted that month (in each ICU), multiplied by 100.

Moreover, we calculated the "incidence density" by month (new cases of KOXA48 divided by the sum of days of stay of all patients in that month, multiplied by 100): incidence/100 person-day (Inc/100p-d).

The microbiological method, was based on clinical or surveillance samples:

Clinical Samples: 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 all the antibiotics tested following the Clinical and Laboratory Standards Institute (CLSI) guidelines. Tigecycline minimun inhibitory concentrations (MICs) were evaluated according to the interpretative criteria of the Food and Drug Administration (USA) (FDA). Extended-spectrum β-lactamases (ESBL) production was confirmed by E-test ESBL strips (bioMérieux) and carbapenem MICs 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.

Surveillance Samples: These, were cultured in MacConkey agar supplemented with 4 mg/L cefotaxime. Disc diffusion and modified Hodge test were performed on all *Enterobacteriaceae* isolates to identify ESBL, plasmid-mediated AmpC, and carbapenemase production.

We mapped the KOXA48-colonized or infected patients on the hospital floor plan. If clonal transmission was suspected, molecular typing of the KOXA48 was performed and carbapenemase genes were confirmed by PCR, using the DiversiLab (bioMérieux) system. The multilocus sequence typing (MLST) —"sequence type"—was determined according to the Institute Pasteur scheme (http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html). Clone-specific real-time PCR for *K pneumoniae* ST11 and ST405 was used for rapid typing.[26]

The bundles recommended to control the KOXA48 outbreak were similar to bundles used to control of other outbreaks,[24] and can be considered an adaptation of those described in the CDC 2012 guide:[13]

(a) Early detection and implementation of contact precautions, with emphasis on hand hygiene with alcohol solutions and surface disinfection, after evaluation of the efficacy of disinfectant and alcohol solution, against the epidemic strain.
(b) Cohorting the cases, grouping them in 1 specific zone of the NICU or PICU. In other departments they were in individual rooms; no changes were necessary.
(c) Cohorting the HCW, especially nurses. In the first month, physicians were also dedicated to the KOXA48-cases, but after that time, they shared in the care of other non-KOXA48 patients.
(d) Visits from outside specialists to neonates were restricted.
(e) Flagging the patient's clinical history with a sheet stating the contact precautions for when the child was taken out of the unit for clinical tests, etc. This same flagging sheet was used when these children were readmitted to the hospital during 2013 to 2014.
(f) Body washing (1/day) was done with 4% chlorhexidine soap rinsing the skin well afterwards (if they were not newborns, because this soap is irritating to the skin of a newborn baby). Newborns >15 days were bathed (1/day) with a 0.5% chlorhexidine aqueous solution.

2.3. Statistical methods

Monthly results of incidence or prevalence were compared by Mann–Whitney *U* test, taking as independent variables, ICU (NICU vs PICU) and year (2012 vs 2013–2014).

3. Results

All the tested microorganisms "in vitro" were susceptible to the 5 alcohol based solutions used, at 20 or 30 seconds, indicating a destruction of >4 $\log_{10}$ of KOXA48 or control strains (Table 1). Consequently, it was not necessary to change Alcoaloe as the hand antiseptic.

However, the surface disinfectant of our hospital (Sanitbio), had only limited activity (<1 $\log_{10}$ of destruction) against the epidemic strain (Table 1) although it was efficient against the strain-control ATCC *K pneumoniae* (>4 $\log_{10}$). We, therefore, changed it or reinforced its efficacy with another product so as to obtain significant destruction of the epidemic strain. Due to the great efficacy of 70% 2-isopropyl-alcohol (>4 $\log_{10}$ of KOXA48 destroyed, similar to 1000 mg/L sodium hypochlorite in our surface test), we decided to use a double application on surfaces: first, Sanitbio (organic material elimination) followed by 70% 2-isopropyl-alcohol (destruction of KOXA48).

Figure 1 describes the time-distribution of incident cases (in the NICU or PICU), of colonization or infection by microorganisms with OXA48 genes isolated during 2012 to 2014 (no cases before 2012). Tables 2 and 3 compare the relative frequency (measured as incidence or prevalence) of KOXA48 in the NICU and PICU.

Table 1

Efficacy (log_{10} reduction) of divers antiseptics or disinfectants on *Klebsiella pneumoniae*: ATCC or OXA48 recently isolated from patients.

Products	Germ-carrier	*K pneumoniae*	Log_{10}: Initial	Final	Reduction
Antiseptics					
Daromix	sim.skin*	ATCC	4.3	0***	>4
	sim.skin*	OXA48	4.8	0	>4
Sterillium	sim.skin*	ATCC	4.3	0	>4
	sim.skin*	OXA48	4.8	0	>4
NDP-derm	sim.skin*	ATCC	4.3	0	>4
	sim.skin*	OXA48	4.8	0	>4
Alcoaloe	sim.skin*	ATCC	4.3	0	>4
	sim.skin*	OXA48	4.8	0	>4
2-IPA** (70%)	sim.skin*	ATCC	4.3	0	>4
	sim.skin*	OXA48	4.8	0	>4
Disinfectants					
Sanitbio (0.5%)	glass	ATCC	5	0	>4
	glass	OXA48	4.7	3,8	<1
Sodium hypochl (1000 mg/L)	glass	ATCC	5	0	>4
	glass	OXA48	4.7	0	>4
2-IPA** (70%)	glass	ATCC	5	0	>4
	glass	OXA48	4.7	0	>4
Sanitbio (0.5%)					
+2-IPA** (70%)	glass	ATCC	5	0	>4
	glass	OXA48	4.7	0	>4

ATCC = American type culture collection, NDP-derm = N-duopropenide, ethanol.
* sim.skin = similar to skin.
** 2-IPA = 2 isopropyl-alcohol.
*** If 0 FCU consider "0" log_{10} (do not $-\infty$).

Incidence (expressed as cumulative or density) was greater in NICU than PICU in 2012, and in both units, in 2012 than 2013 to 2014, but due to great number of "0" in monthly results, is not necessary to apply any statistical test to conclude the existence of differences between years or Units. But monthly results of prevalence can be compared by Mann–Whitney *U* test obtaining that the prevalence was different ($P < .01$) between both Units, but not between 2012 and 2013 to 2014 ($P > .2$).

All were *K pneumoniae* and belonged to the same strain (ST11). These bacteria have genes for carbepenemase, cephalosporinase, and porin-modification, and are only susceptible to amikacin, colistin, and fosfomycin.

The patients were negative for these KOXA48 on admission (first screening) and became positive after 1 or more weeks (median, 23 days, seen in successive screenings, or noted in clinical samples, taken on suspicion of infection). In most cases, identification of KOXA48 was unexpected as patients were asymptomatic.

Due to these systematic screenings, antibiotic use was predominantly oriented by antibiogram, and not by empiric treatment. Moreover, both intensive care units (NICU and PICU) have a restriction policy for antibiotics.

The time distribution of cases (Fig. 1 and Table 2) indicates an outbreak of KOXA48 in 2012 (no cases before this year) that was eradicated before the end of the year and was not transmitted to new patients in 2013 to 2014, despite readmission of some old colonized cases (Table 3).

The outbreak was mainly in the NICU with 16 cases (along with another case, detected in the PICU) and started from a child colonized in February 2012 from his mother, who in turn had been colonized by another patient during her stay in the postpartum Observation Unit. This child infected another one and isolation precautions were taken for both cases. We thought the outbreak was over when the index case was discharged and 4 negative rectal controls had been taken from the second case.

However, in the following week, the second NICU case repositivized, and a third case appeared in the same week, followed by others in the subsequent weeks. This occurred despite the control measures taken (contact precautions and cohorted cases, but not HCW cohort). Case numbers increased until the month of August (peak of incidence: 8.8%), when there were 4 new cases. This may have partially been due to the hiring of new HCW (personnel for summer vacation substitutions) who may not have had sufficient training in contact precautions. In September, we concluded that the measures already taken had to be strengthened, given the failure of the cohorting and contact precautions. The NICU was closed to new patients for 15 days; we created a cohort of colonized or infected cases and a cohort of HCW, who only treated these patients. The entry of external

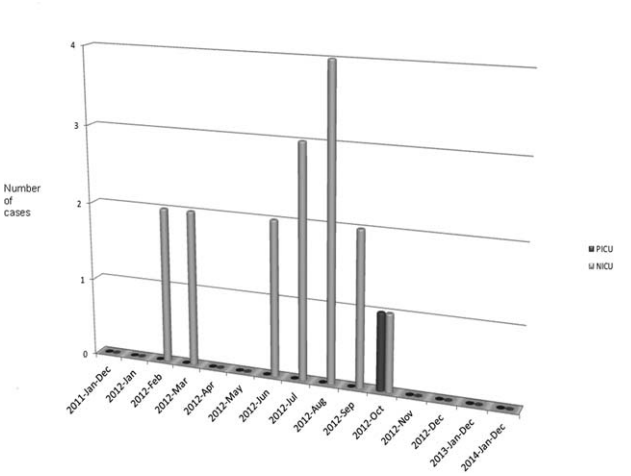

Figure 1. OXA48-*K pneumoniae* outbreak in NICU and PICU (2012–2014). NICU = neonatal intensive care unit, PICU = pediatric intensive care unit.

Table 2

Incidence (cumulative) and density of incidence (by 100 person-day) of *K pneumoniae* OXA48-cases in a NICU and a PICU in a children-hospital, during 3 years.

Time	NICU Incidence %	NICU Inc/100p-d	PICU Incidence %	PICU Inc/100p-d
Jan-2012	0.0	0.0	0.0	0.0
Feb-2012	5.4	0.3	0.0	0.0
Mar-2012	3.8	0.3	0.0	0.0
Apr-2012	0.0	0.0	0.0	0.0
May-2012	0.0	0.0	0.0	0.0
Jun-2012	4.1	0.3	0.0	0.0
Jul-2012	6.0	0.5	0.0	0.0
Aug-2012	8.8	0.7	0.0	0.0
Sep-2012	5.7	0.4	0.0	0.0
Oct-2012	2.3	0.2	1.5	0.3
Nov-2012	0.0	0.0	0.0	0.0
Dec-2012	0.0	0.0	0.0	0.0
Jan–Dec 2013	0.0	0.0	0.0	0.0
Jan–Dec 2014	0.0	0.0	0.0	0.0

Jan–Dec 2011: 0 cases in NICU or PICU.
Inc/100p-d = density of incidence (by 100 person-day).
NICU = neonatal intensive care unit, PICU = pediatric intensive care unit

Table 3

Prevalence (%) of *K pneumoniae* OXA48-cases in a NICU and a PICU in a children-hospital, during 3 years.

Time	NICU	PICU
Jan-2012	0.0	0.0
Feb-2012	5.4	0.0
Mar-2012	5.8	0.0
Apr-2012	6.0	0.0
May-2012	3.4	0.0
Jun-2012	6.1	0.0
Jul-2012	10.0	0.0
Aug-2012	20.0	0.0
Sep-2012	20.0	0.0
Oct-2012	9.3	4.4
Nov-2012	10.5	3.4
Dec-2012	4.7	2.3
Jan-2013	4.9	0.0
Feb-2013	3.7	1.2*
Mar-2013	16.6*	0.0
Apr-2013	10.7*	0.0
May-2013	0.0	1.4*
Jun–Oct-2013	0.0	0.0
Nov-2013	0.0	1.2*
Dec-2013	0.0	0.0
Jan–May-2014	0.0	0.1*
Jun–Dec-2014	0.0	0.0

Jan–Dec 2011: 0 cases in NICU or PICU.
NICU = neonatal intensive care unit, PICU = pediatric intensive care unit.
* Prevalence due only to readmission of old cases.

HCW from other specialties was restricted (a maximum of 2 HCW, for each specialty, saw to 1 patient) and we stressed the need to improve compliance with contact precautions in health education sessions. This "bundle" of measures was completely introduced in the middle of September.

In September, we only had 2 new cases (incidence 5.7%) and only 1 more new in October (incidence 2.3%). The cohort of neonatologist physicians was no longer maintained, but the nurse-cohort was. From November no new cases were detected, even though the children remained in the NICU for a long time (some for several months). These children had many family problems and, unsurprisingly, the older children/babies needed more attention than other neonates, but there was no more transmission of these organisms.

Several of the KOXA48-patients required readmission to another unit of our Children's Hospital during 2013 to 2014, and they produced no transmissions. All these patients were previous cases that remained colonized (prevalent cases, Table 3), therefore the risk of transmission persisted if appropriate precautions were not correctly followed.

Last, 1 KOXA48-patient had been transferred from the NICU to the PICU, and another child (who had a cancerous process) coincided with him for a week in the PICU. The rectal culture taken the day before the PICU-Oncology transfer from that patient was positive for KOXA48. However, there were no transmissions to other patients in the PICU or in Oncology. Moreover, in the rest of this children's hospital, none infected or colonized cases were detected during 2012 to 2014.

Overall, the outbreak affected 17 children, 9 men. Most were only colonized (14 of the 17 cases) and were asymptomatic. Diagnosis was made due to routine surveillance procedures and the median number of days from admission to detection of KOXA48 was 23 days (inter-quartil-range [IQR] 11.5–55.5 days). Four of these children died (23%), but this was due to their underlying health process, not to KOXA48.

4. Discussion

An experiment with 11 KOXA48 strains, determined the environmental resistance of these microorganisms to desiccation and their persistence on surfaces: KOXA48 resisted a maximum of 21 days (unpublished data). This environmental resistance may explain part of the epidemic diffusion, mainly if surface disinfection is not adequate. A resistance to alcohol solution by this epidemic strain is improbable[27] but this possibility must also be examined. We demonstrated with the initial strains isolated from our patients, that our previously selected alcohol solution (Alcoaloe) can be used without problems, but other measures, like surface disinfection, employed an inefficient disinfectant (a diluted quaternary ammonium). After supplementing the use of this product with isopropyl-alcohol, the KOXA48 strain was susceptible to the antiseptic and disinfectants considered, in our contact precautions. Moreover, we demonstrated that antiseptic or disinfection tests must be performed with the epidemic strain, and not only with the ATCC control strain, which is normally quite sensitive, and not representative, of a patient's micro-organisms.[19]

Moreover, a daily bath of KOXA48-cases with chlorhexidine (aqueous-0.5% in neonates >15 days old and soap-4% in children >2 months old) has not originated any adverse event in our colonized or infected children. This measure has also been used in other outbreaks in our hospital without observed adverse events.

It is interesting that in children the KOXA48 outbreak was due to the transmission of a single strain (ST11), which remained there since it began in February 2012. This strain of *K pneumoniae* has appeared frequently in adults in our hospital (during 2012–2014), as well as hospitals in other countries, but that microorganism was not isolated in any child of our hospital previously, according to our system of continuous epidemiological surveillance. The outbreak in our hospital may be due to 3 causes:

(1) Errors in contact precautions
(2) The high environmental resistance of this ST11 strain (it was the most resistant of the different KOXA48 strains studied in our earlier study), and
(3) The poor surface disinfectant capacity of the product previously employed in our hospital (a diluted quaternary ammonium, which we tested and saw only eliminated a little over 1 $\log_{10}$ of ST11 on a surface).

Then, when we improved the compliance of the contact precautions (by health education sessions and surveillance of Preventive Medicine Nurse), supplemented the above disinfectant with another to improve microorganism elimination (performing a second disinfection with 70% isopropyl alcohol), and make cohorts of children and HCW, in addition to early detection of colonized patients, the disappearance of the outbreak in Children's Hospital was obtained. For these causes, although in 2013 to 2014 many cases in adults of the Hospital La Paz were diagnosed and we had readmission of KOXA48-children, we had no new cases with this microorganism.

However, the double application may not always be performed correctly on all surfaces and there may be areas where the quaternary ammonium alone is used, resulting in a non total destruction of the bacteria. Because of this we looked for a

product that was both an effective cleanser and disinfectant and required only 1 application.

In 2014 we are using a chlorinated compound (2% Sprint-clorado, with 4000 mg/L of Cl-free) with a dual cleansing and disinfection effect (destruction of >4 $\log_{10}$ of microorganisms on the same standard surface where the environmental persistence was assessed). Hopefully, the new product, along with greater discipline in fulfilling contact precautions, will reduce the transmission of these KOXA48 microorganisms. However, due to a possibility of irritant vapors after application of this product, we do not indicate it for newborns. In these patients's environment, the previous double disinfectant application is still performed.

5. Conclusions

Infected children are the tip of the iceberg (3/17 = 17%) of KOXA48 incidence. It is also necessary to identify the cases colonized by these bacteria by a systematic surveillance.

In the beginning of an outbreak, is necessary to study (in vitro) the susceptibility of the epidemic strain to antiseptics and surface disinfectants, changing the products used, if necessary.

The measures taken (early detection, cohort of children and HCW, contact precautions, etc.) must be thorough, immediately after microbiological diagnosis, in colonized and infected cases, because if the bundle compliance is not total, the outbreak will not be controlled, as occurred during summer of 2012 in NICU. After, the measures against the transmission of microorganisms were reinforced and the outbreak was eradicated, despite readmission of some old colonized cases in our children's hospital, during 2013 and 2014.

Acknowledgments

To Laboratory Technician Mayca Uriarte-Martinez.

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