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Research Article

A Case and Negative-Control Study of Neonate Risk Factors for Colonization or Infection by VIM-*K. oxytoca*

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Abstract

Background: We study the risk factors of the first outbreak of VIM-carbapenemase-*K. oxytoca* in a NICU.

Methods:

Epidemiological surveillance:

a) Systematically:

a.1) weekly screening cultures of neonate microbiota or

a.2) microbiota studies during the NICU admission if the patient had a history of previous colonization by VIM-*Enterobacteriaceae*.

b) Clinical cultures, done only if infection was suspected.

The risk factors for colonization, or infection, by VIM-*K. oxytoca* have been studied through a "case and negative-control" design.

Results: We identified 20 VIM-*K. oxytoca* cases. The most only presented colonization, but four showed infection. There were VIM-*K. oxytoca* bacteria in the sinks (drains) but there were not genetically the same as those in the patients, who showed three different strains. The controls were the neonates admitted in the same period of cases in whom the microbiota-study no had detected carbepenemase-producing bacteria (negative-control). There were 110 controls.

The Multivariable logistic regression included only three variables for the colonization/infection by VIM-*K. oxytoca*: one was protective and two, risk factors:

1) Neonate "susceptibility", evaluated according to the time from NICU admittance (OR = 41, during the first 15 days of life).
2) Duration of central catheter use (OR 1.2/day).
3) Birthweight (OR 0.89/100g).

Conclusions:

- The risk factors of VIM-K. oxytoca colonization/infection in a NICU, were studied using a "case with negative-control"design. The number of patient was 20 cases and 110 controls.
- This study design has allowed us to identify by multivariate analysis two risk factors (time from NICU admittance during the first 15 days of life, and duration of central catheter use) and one protective factor (birthweight).

Keywords: *VIM-K. oxytoca; NICU; Case and Negative-Control Study*

Introduction

The frequency of carbapenemase-producing microorganism isolation in tertiary hospitals has been rising since 2007 [1], particularly *Enterobacteriaceae* in healthcare associated infections such as septicaemia, ventilation-associated pneumonia, urinary tract or surgical site infections [2,3]. Molecular biology techniques have detected antibiotic resistance genes like carbapenemase Ambler types A, B and D [4]. The Class B contains divers genotypes as the VIM type.

When a new patient is colonized or infected by VIM-microorganisms, it is necessary to determine whether this is due to microorganisms being carried by healthcare workers, or whether infection comes from microorganism reservoirs that are difficult to clean during the inter-patient room cleaning/disinfection [5-7]. These reservoirs have sometimes been seen with hydrophilic bacteria like *P. aeruginosa*, *B. cepaciae*, or *Klebsiella oxytoca* with VIM-carbepenemase (VIM-Kox). These bacteria have been detected in outbreaks in different tertiary hospitals and are associated with sink contamination [8-10].

In this paper we report the first VIM-Kox outbreak in a NICU at a tertiary children's hospital, studying the risk factors for colonization/infection of these microorganisms through a "case and negative-control" (nested in a cohort) design.

Material and Methods

La Paz Children's Hospital is a tertiary hospital with one NICU. Since 1985 the control of hospital infection, is performed by one medical epidemiologist (part-time) and one nurse epidemiologist (dedicated full time).

Epidemiological surveillance for infection or colonization by multi-drug resistant microorganism is performed in two ways: systematically, with an active surveillance methodology using weekly screening cultures taken from all children admitted to the NICU, or a second surveillance method employing clinical cultures that are performed if infection is suspected or if the patient has a previous multi-drug resistant microorganism colonization history.

All these data together clinical characteristics of each patient (clinical chart), were collected prospectively in epidemiological records of Preventive Medicine Service. After, we used these epidemiological chards for to analyse the main risk factors of colonization/infection by these *Enterobacteriaceae* and communicate to NICU staff the results.

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 *K. oxytoca* and other VIM-*Enterobacteriaceae* genera.

The "controls" were the children (admitted in the same period of cases) in whom the microbiota study (performed at least once) had shown no carbepenemase-producing bacteria.

Control measures: Bundles recommended for controlling VIM-*Enterobacteriaceae* were adapted [11-13] from those described in CDC 2012.

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.

Statistical method: We collected information from the clinical histories of each patient (infected, colonized or negative-controls) with respect to different variables present on admission (prematurity, weight, sex, etc.), as well as others that arose during their stay in the NICU (arterial or venous catheter use, surgery, etc.).

"Number of days at risk of being a VIM-Kox-case": time between entrance to our NICU until diagnosis of VIM-Kox in some clinical or epidemiological sample from a patient. For each control patient, the risk-time was the entire NICU stay.

Other time-variables were: duration of central venous catheter, mechanical ventilation or antibiotic use. All the days these techniques were needed, for the controls, and only until the time of colonization or infection, in the VIM-Kox cases, were counted.

In addition to the variables obtained from the infants´ clinical histories, "ecological variables" were included: number of neonates with VIM-Kox diagnosed in the first, second, third, etc weeks after NICU admittance.

All variables were analyzed using the SPSS-19 program (SPSS Inc., Chicago IL). We performed bivariate and multivariable analyses: VIM-Kox vs. Controls. Quantitative variables were analyzed with ANOVA and Bonferroni post-hoc tests. For variables that did not follow a normal distribution, nonparametric tests (Kruskal-Wallis or Mann-Whitney) were used.

Qualitative variables were studied with Pearson's chi-squared test. To determine risk factors for colonization/infection, multiple logistic regressions were performed, controlling for different confounders, and using the Hosmer-Lemeshow test for goodness of fit. The cut-off point for the variables entering into logistic regression was $p < 0.2$ in the bivariate analysis.

Results

The VIM-Kox NICU outbreak began in the first week of February and ended in May of the same year (2014). In all, the NICU had 20 cases of VIM-Kox (4 of them had VIM-Kox and VIM-Seratia). Of the VIM-Kox cases most were only colonizations but 4 also had infection (3 pneumonias and one case of conjunctivitis).

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). These bacteria VIM-Kox were genetically different from those in the neonates, who showed three different strains.

The epidemiological study was designed as a "case with negative control", nested within a cohort. In the first instance, cases were NICU patients with VIM-Kox. The three cases admitted to the unit from other hospitals already carrying VIM-Kox were excluded. There were 110 controls.

Controls differed from cases in frequency (16% vs. 30%) and type of Health Associated Infection. The cases predominantly showed respiratory infection (6 children, 3 with VIM-Kox and the other three with other microorganisms) but the controls predominantly showed septicemia (8 neonates, due to coagulase-negative *Staphylococcus*). Infections like conjunctivitis (1 case with VIM-Kox), viral gastroenteritis, viral respiratory infections (i.e. Respiratory Syncitial Virus), etc. were also detected.

Other variables showing significant differences in the bivariant analysis (Tables 1 and 2) included opiate abstinence syndrome, surgery, length of NICU stay and duration of central venous catheter use. Curiously, VIM-Kox detection (for cases) occurred around the half-point of the patient's stay in the NICU (median 15 days vs 47 days for global stay) and the controls had an overall NICU stay that was as long as the time needed for colonization/infection by VIM-Kox (median 15 days).

Variable	Control		VIM-Kox case
Sex = male	56%		50%
Birthweight < 1000g	7.3%		15%
Prematurity	49%		45%
Respiratory distress	55%		55%
Cardiovascular malformations	30%		45%
Other malformations	25,5%		25%
Withdrawal opiate syndrome	14%	--*--	40%
Surgery	23%	--*--	55%
Exitus	3.6%		10%
Cases in 1st week†	39.1%		45%
Cases in 2nd week†	49.1%		70%
HAI	16%	--*--	30%
Resp infection	1.8%	--*--	15%
Septicemia	8%		0%
"other" HAI	6%		15%
Previous Antibiot††	77%		80%
-B-Lact+Agl	49%		30%
-B-Lact+Agl+Macr	10%		25%
-other Antibiot	18%		25%

Table 1: *Qualitative variables in cases and negative-controls.*

Notes: *Resp: Respiratory; HAI: Healthcare Associated Infections; †: Weeks After NICU Entrance; ††B-Lact: B-Lactamics; Agl: Aminoglycosides; Macr: Macrolides; --*--: $p < 0.05$*

Variable	Control			VIM-Kox case	
	Median	X ± SE		Median	X ± SE
Global NICU- stay (days)	14	23 ± 2	--*--	36	42 ± 8.2
Global Neonate stay (days)	15	26 ± 2	--*--	47	54 ± 7
NICU-stay to VIM (days)	15	26 ± 2		15	25 ± 5
Birthweight (g)	2620	2504 ± 81		2345	2204 ± 220
Days with Antibiotherapy	7	6.5 ± 0.6		7	9.1 ± 1.8
Days with Central Venous Catheter	3	5.8 ± 0.7	--*--	14	15.8 ± 2.7
Days with Mech. Ventilation	0.5	2.1 ± 0.5		1	7.8 ± 3.8

Table 2: *Quantitative variables in cases and negative-controls.*

Notes: *--*--: p<0.05; MW: Mann-Whitney; X ± SE: mean ± standard error; Mech: Mechanical*

Antibiotic administration was similar (nearly 80%) in cases and controls, whether qualitative (type of antibiotic) or quantitative (number of days until VIM-Kox isolation in cases and total days of administration for controls).

Death occurred in 3.6% of the controls and 10% of the cases, but was unrelated to the VIM-*K. oxytoca* infection.

Multivariable analysis (Table 3) showed "central venous catheter" duration as a predictive variable for VIM-Kox colonization/infection with an OR of 1.2 per day (i.e. 5 days' catheter placement would produce a 2.3 OR and at 10 days the risk would be 5.3); the main variable, "VIM-Kox susceptibility" was distributed into three classes based on a recodification of the "time at risk" (i.e. time to bacteria identification in the cases or the entire NICU stay for controls). Susceptibility can be "high" with an OR = 41 (in the first fortnight after admittance to NICU), "medium" with an OR = 8 (second fortnight in the unit), or "low" (a stay of longer than one month), which is the reference category (OR = 1).

VIM-Kox					
Variable	β	SE-β	p	OR	OR -95% C.I.
Susceptibility to KoVIM					
Media/reference	2.13	1	< 0.05	8.4	1, 73
High/reference	3.71	1.15	< 0.01	41	4.2, 393
C. VenCat-time (days)	0.18	0.04	< 0.01	1.2	1.11, 1.3
Birthweight (Hectograms)	-0.11	0.04	< 0.01	0.89	0.82, 0.97
Constant	-3.25	1.03			
-2Log-likelihood 81.23 (NS); Area Under the Curve ROC: 0.844					

Table 3: *Multivariable logistic regression (case vs negative-controls).*

Notes: *SE: Standard Error; CI: Confidence Intervals; C. VenCat-time: Central Venous Catheter-Time*

The equation also included a variable of OR < 1 (protection factor): "birthweight" (OR=0.89 per each Hectogram increase in weight).

The logistic regression fit was good (Area under the curve ROC = 0.844), showing no difference between observed and model-predicted values.

Discussion

Several studies [8-10] 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´s VIM-Kox were different from those in the neonates. Nevertheless these 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 30 for colonized patients [13]), and were enacted as soon as possible, producing a large reduction in incidence as soon as the measures were in place.

The study design using a case with negative control (nested in a cohort) is much more appropriate for the study of VIM-Kox colonization or infection than the classic case and control design [14,15] since patients with asymptomatic processes like these would have been considered controls in many instances, unless the question of whether or not they had been colonized by the VIM-microorganisms had been examined. These patients are very similar in most of the studied variables. A multivariable analysis was performed to identify the VIM-carbepenemase associated variables.

This analysis revealed the classic healthcare associated infections risk factors for infants [13,16] like "duration of central venous catheter" (before isolation of VIM-Kox for the cases and total catheterization time for the controls) and "low birthweight". Other variables like respiratory distress, opiate abstinence syndrome, mechanical ventilation, antibiotherapy, etc., which would theoretically imply greater propensity for colonization or infection by exogenous microorganisms from other patients, the environment or healthcare workers, were not accepted in the final multivariable equation, because most of the information supplied by the prediction of being a "case" was already usually included in the key variable in this study: the amount of time at risk for colonization or infection by the VIM-Kox. For the cases, this time was the number of days passed from admittance until VIM-bacteria isolation (the rest of their stay is not considered), for controls their risk time was their entire NICU stay since they could have acquired the bacteria at any time during that stay. This is the proper way to calculate this, but in other studies, instead of applying this variable, they study global stay, which is also longer in cases, so they would consider some days as risky even when there was no longer any "risk of colonization or infection" because the patient was already a case and this would consequently distort the resulting predictive model.

By directly studying the time at risk for acquiring VIM-Kox, time is seen to be a protective factor since risk is reduced the longer the patient is in the NICU. The only explanation for this would be some sort of "maturation", like inspecific immunity, stabilization of the intestinal microbiota, thickening of the epidermis, etc. [17-20], that would give the neonate greater resistance to colonization by exogenous microorganisms with every day that passes.

Therefore we have considered it best to transform the time variable into another that is more indicative of "degree of susceptibility" and would have an OR > 1, like the classic risk factors. We have consequently codified susceptibility as "high" (first fortnight after admittance), "medium" (second fortnight) and "low" (at one month after being admitted, and this is the reference category). Thus, the ORs are greater than one and an exponential decrease in susceptibility can be demonstrated; for example, in the first fortnight susceptibility is 41 times greater than a month after admittance to the unit, and, in the second fortnight, the risk is 8 times greater than at one month after admittance. Curiously, studies on the maturation of intestinal microbiota, which affect immunity [19,20] describe the first 15 days of life as necessary to begin to stabilize these microbiota, and this time point also corresponds with the mathematical model found here with a much higher OR for this period than the OR for any of the other factors determining VIM-Kox colonization risk.

Conclusion

- A VIM-Kox colonization or infection outbreak in a neonate NICU is described. The outbreak was relatively brief. The source of these microorganisms was other patients.

- The used design was a case - negative control study (nested in a cohort).
- The risk factors were: "degree of susceptibility" (the most important with an OR that may reach > 40 in the first fortnight of the NICU stay) and "duration of central venous catheter use" (as an example, at 10 days of use, OR would be > 5).
- A protective factor, "birthweight" has also been detected; for every 100 gram increase in birthweight, the VIM-Kox colonization or infection risk is multiplied by 0.89.

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Author Contributions

RH and FO conceived and designed the research. GR performed the microbiological analysis. RH and SG 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).

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Conflicts of Interest Statement

None.

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