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Full Length Research Paper

If you are looking for, you can find endemic bla-VIM gene microorganisms, in Children's Hospitals

Herruzo R[1], Ruiz G[2], Burgos C[3], Perez-Blanco V[3], Gallego S[4], Mora E[3], Omeñaca F[5]

[1]Catedratico de Medicina Preventiva y Salud Publica. Departamento de Medicina Preventiva y Salud Publica y Microbiologia, Universidad Autonoma de Madrid.
[2]Medico Adjunto. Servicio de Microbiologia Hospital Universitario La Paz. Madrid.
[3]Medico Adjunto. Servicio de Medicina Preventiva. Hospital Universitario La Paz. Madrid.
[4]Enfermera del Servicio de Medicina Preventiva. Hospital Universitario La Paz. Madrid
[5]Jefe del Servicio de Neonatologia. Hospital Universitario La Paz. Madrid.

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Background: Time-trend of VIM carbapenemase-producing microorganisms in a tertiary children's hospital during two years, as well as the preventive measures taken in place, are been described. Methods: Epidemiological surveillance for these microorganisms was performed in two ways: a) systematically, (weekly screening cultures taken from all children admitted to the two ICUs, or every 15 days for Oncology and Transplants Services; b) via clinical cultures done if infection was suspected, or (at admission) if the patient had a previous colonization history of VIM microorganisms. The bundles recommended for control of the VIM strains were adapted from those described in CDC 2012. The evaluation of the environment resistance and susceptibility to antiseptics or disinfectants were implemented using standard germ-carriers. Results: These VIM microorganisms survive 35 days on germ-carriers in dry conditions. They were susceptible to alcohol and moderately resistant to diluted quaternary ammonium. The incidence of VIM cases was 0.9% (2012) to 0.77% (2013), but the prevalence was higher, similar to an endemic situation. VIM microorganisms show a very temporal, geographical and etiological dispersion (*K. pneumoniae*, *E. coli*, *P. aeruginosa* and *Citrobacter*). Most of the 137 incident cases detected were colonization (75%, but in neonates this increases to 90%). Control measures for these microorganisms were better followed in neonates- ICU (their incidence dropped from 5.3% to 1.9%) than in the rest of hospital (0.6% and 0.7% respectively) in the same period. Conclusions: -There were temporal, geographical and etiological dispersion of VIM microorganisms. -Generally only produced colonization- These difficulties originated that the indicated control measures are not totally met. -Consequently, VIM-microorganisms have become endemic in our hospital.

Keywords: Endemie; bla-VIM-microorganisms; Children's Hospital

Running title: bla -VIM microorganisms in Children's Hospital

INTRODUCTION

The frequency of carbapenemase-producing microorganism isolation in tertiary hospitals has been rising since 2007 (Tato et al, 2007), particularly *K. pneumoniae* in urinary tract infections, surgical sites, septicemia and ventilation-associated pneumonia (Zaoutis et al, 2005; Gupta et al, 2011; Walsh et al 2011; Canton et al, 2012; Paño-Pardo et al, 2013). Molecular biology techniques have detected antibiotic resistance genes such as *carbapenemase* Ambler types A, B and D (Nordmann and Carrer , 2010). Among others, the Class B or metallobetalactamases (which contain zinc in their active site), are, the VIM and NDM types, described in Verona and New Delhi, respectively.

They all produce beta-lactamases and some also present porin modifications, another mechanism that produces antibiotic resistance. These mechanisms can be reversible, since bacterial porin modification may also decrease growth and substrate utilization. Therefore they may be eliminated in the intestine through competition with microbiota without these bacterial resistance mechanisms for bacteriocins, nutritional competition, etc. (Ruppe and Andremont, 2013), if we manage to reduce antibiotic pressure and have sufficient time , often years (Zimmermann et al, 2013) .

It is essential to reduce transmission between patients, both from direct contact (patient-to-patient) and from 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 they often require behavioural changes that are poorly followed by HCW (especially physicians), or are soon forgotten if not reinforced regularly.

Finally, control measures should also affect the cleaning and disinfection of surfaces (Nseir et al, 2011; Havill, 2013; Carling, 2013; Herruzo et al 2014) in the patients` environment, such as sinks, faucets, doorknobs, etc., which may be reservoirs for these multidrug resistant microorganisms (MDRM). With regard to visitors, we must balance the need for family support and the isolation precautions needed in each case, so as to not unduly complicate the patient's treatment.

In this paper we describe the time-trend of VIM carbapenemase-producing microorganisms in a tertiary children's hospital, during two years, as well as the preventive measures taken.

*Corresponding Author's Email: rafael.herruzo@uam.es

MATERIAL AND METHODS

La Paz Children's Hospital is a tertiary hospital with two Intensive Care Units: Pediatric (PICU) and Neonatal (NICU). Moreover, this hospital offers all the pediatric specialties: general paediatrics; cardiologic, digestive, orthopaedic, surgery etc.; dialysis, oncology, infectious diseases and transplants. Since 1980 monitoring and control of hospital infection is performed by one medical epidemiologist (part-time) and one 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. (Herruzo et al, 2001; Herruzo et al, 2014) as well as endemic infections associated with the four main causes of hospital infection: surgical site infection, urinary tract infection, ventilator-associated pneumonia and central venous line-associated sepsis (Ruza et al 1998).

Different MDRM 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 surveillance for infection or colonization by these microorganisms was performed in two ways: systematically, with an active surveillance methodology using weekly screening cultures taken from all children admitted to the two ICUs, or every 15 days for the rest of the patients (oncology and transplant services); the second surveillance method was via clinical cultures that were performed if infection was suspected, or if the patient had a previous colonization history. MDRM obtained from any patient sample (colonization or infection) determined the patient`s classification as an "MDRM-case".

Microbiological method

A) **In vitro studies**

Materials:

a) Microorganisms: Collected from clinical samples or faecal microbiota of La Paz University Hospital patients:
(I) Six strains of microorganisms with VIM-carbepenemase (One *K. pneumoniae*, two *Klebsiella oxytoca*, two *Enterobacter cloacae* and one *Serratia marcescens*)
(II) Controls:
Three ATCC strains: *K. pneumoniae* 13883, *E. coli* K 12, *P. aeruginosa* 10145.
b) A surface-germ-carrier: a standard-sized, easy-to-manipulate surface model (Herruzo et al, 2014). Glass cover-slides, sized 12 × 35 mm The number of bacteria from 10 µL of nutrient broth (after 24 h incubation at 37ºC) or from the germ-carrier contaminated with 10 µL of this broth were very similar (6.65 log10 vs 6.48-6.7), indicating

excellent recovery of the inoculums on theses germ-carriers.
c) A skin-germ-carrier model, as described in another paper (Herruzo et al 2010).
d) Antiseptic and disinfectants in use in our hospital during 2012-3
Hand antiseptic: AGB Lab. Alcoaloe® (0.6% chlorhexidine + alcohol+ aloe vera)
Surface disinfectant: Lab Proder-Pharma. Sanit-bio® Used diluted 200-fold (0.5%) (Composition per 100ml: 1.6g benzyl chloride, C12, C18 alkyldimethylammonium, 1.5g chlorine-didecyldimethylammonium chloride and 1.6g benzyl-C 12 alkyl dimethyl C14 and <5 g of anionic surfactants).
Isopropyl-alcohol: 70% (dilution from Panreac 96º isopropyl-alcohol) Sprint-chlorado ® : Lab Diversey. Used at 2% dilution: 4000 ppm of free cl^-).

METHODS

1) Environmental resistance study of bacteria with VIM carbepenemases (or ATCC strains), on the germ-carrier surface

We chose a standard surface (the glass cover-slides described in section b of Materials), which was cultured for 48 h in Todd Hewitt broth (Difco) at 37°C. Ten microliters of each microorganism (normally these inoculums/a are between 6 to 7 log10) were poured on the 12 germ carriers. After 1 h (during which time the inoculum dried, and this sample is our control), we used flame-sterilised tweezers to extract the germ carriers and deposit them in a trough. The trough was then covered with a glass and left until processing and seeding on different plates, in order to calculate the number of microorganisms surviving after different time periods.
We studied the following times: +1 h (day 0=control), 24 h (day 1), 48 h (day 2), daily until the 7th day, and then weekly on the 14th, 21st, 28th and 35th days. We plated 12 germ carriers for each microorganism, and we calculated the number of surviving colony-forming units (CFUs) at each time point as described below.
- We took the germ carrier being studied (for example, at day 1 = 24 h) and immersed it in a test tube with 5 ml of Todd-Hewitt broth and 0.5 g of sterile glass beads. This was centrifuged at 2000 rpm for 1 min to release the surviving microorganisms and disinfectant inhibitor into the culture broth. Next, we extracted two 0.1-ml aliquots and spread each of them over the entire surface of separate McConkey plates, which were then incubated at 37°C for 48 h. Last, we counted the CFUs. As the number of CFUs could be very high, we also seeded two plates with 0.1 ml from 1/10 and 1/100 dilutions.
2) Efficacy of the alcohol solution against VIM-microorganisms and ATCC strains, on a skin carrier model. The method is described extensively in another paper (Herruzo et al, 2010).
3) Evaluation of the efficacy of two surface disinfectants (alone or by successive applications) on VIM-microorganisms (or ATCC strains) on a surface-model-carrier. The model was cover-slides. These carriers were placed on parallel glass rods across a hollow metal container. The glass rods and the germ carriers where sterilized by flame. After cooling, 10 microlitres of a 24h culture at 37ºC of one of the strains used (VIM or controls) were poured on each cover-slide, for each culture and left to dry for 60 minutes. Next we used sterilised forceps to pick up and place one germ carrier between the index finger and thumb taking a microfibre cloth of 3 x 6 cm impregnated with 0.5 ml of one surface disinfectant. This product was applied twice on the carrier. Afterward, the carrier was left for 15 minutes. Next, the germ-carrier was placed with sterile forceps into a 5ml test tube with a proven efficient disinfectant-inhibitor (nutrient broth + 0.5% sodium thiosulfate and 0.5% sodium bisulfite) and 0.5g of sterile glass beads. The tube was centrifuged at 2000 rpm for 1 minute to release the bacteria from the carrier into the broth. We then took three 0.1ml aliquots and seeded them over the whole surface of six McConkey plates, which were incubated at 37ºC for 48h. Last, CFUs were counted.
The controls for this experiment were similar to the above germ-carrier and were treated in a similar way, but using sterile water instead of disinfectant on the microfibre cloth. Because of the large amount of bacteria that are usually obtained, we made 1/100 and 1/10000 dilutions with sterile distilled water, before seeding the 0.1 ml aliquots on the McConkey plates and proceeding to the 48h incubation at 37ºC. Afterward, CFU are counted, and the log10 reduction for each product calculated.
As an exception to the above, only one application of Sanit-bio followed by another with alcohol, were made to evaluate if the two products can be used successively.

B) Surveillance studies in patients:

B.1) 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 any of the antibiotics tested following the CLSI guidelines. Tigecycline MICs were evaluated according to the interpretative criteria of the FDA. ESBL production was confirmed by E-test ESBL strips (bioMérieux) and carbapenem MICs were confirmed by Etest (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.

B.2) Surveillance Samples

These samples were cultured in MacConkey agar supplemented with 4mg/L cefotaxime. Disc diffusion and a modified Hodge test were performed on all *Enterobacteriaceae* isolates to identify ESBL, plasmid-mediated AmpC and carbapenemase production.
We mapped the MDRM-colonized or infected patients on the hospital floor plan. If clonal transmission was suspected, molecular typing was performed and carbapenemase genes were confirmed by PCR, using the DiversiLab (bioMérieux) system.

-B.3) Controls:

The bundles recommended to control MDRM were adapted from those described in CDC 2012 :

a) Early detection and implementation of contact precautions, with emphasis on hand hygiene with alcohol solutions. This has been proven effective in other outbreaks (Herruzo et al, 2001), and destroyed more than 4 $\log_{10}$ of the MDRM from our hospital in only 15 seconds.

b) Cohorting the cases, grouping them in one specific zone of the NICU or PICU. In other areas they were in single rooms; no changes were necessary.

c) Cohorting the HCW, especially nurses. In the first month, physicians were also dedicated to the MDRM-cases, but after that time, they shared their care with other non-MDRM patients.

d) Restriction of use of B-lactam-antibiotics in Services with one o more MDRM-cases.

e) Signalling/Flagging the patient's clinical history with a sheet stating the contact precautions, as for when the child was taken out of the unit for clinical tests, etc. This same signal sheet was used at readmission of these children to the hospital.

f) Body washing was done with 4% chlorhexidine soap (if they were not newborns), rinsing the skin well afterwards. Newborns were washed with an 0.5% aqueous chlorhexidine solution.

g) disinfection of surfaces based on disinfectants with demonstrated efficacy against these MDRM (recently obtained from patients).

Our Epidemiologist-Nurse evaluated implementation of these measures daily, mentioning/commenting any failures in compliance with the HCW. The Medical- Epidemiologist reinforced these daily recommendations with the main worker responsible for doctors and nurses in these hospital areas.

RESULTS

Environmental resistance of VIM bacteria: The experiment with 5 VIM bacteria (*P. aeruginosa, E. cloacae*, *K. pneumoniae*, etc.) demonstrated the environmental resistance of these microorganisms to desiccation and their persistence on surfaces, where they lasted for over a month. However the ATCC strains did not survive more than one week. This environmental resistance may explain part of the capacity of these VIM-microorganisms to become endemic.

The efficacy of the alcohol solution on the skin model was complete (>4 log10 reduction) with all samples of VIM and control-strains used. Thus, we can continue use the same alcohol solution on HCW and visitors' hands.

The double application of quaternary ammonium used in our hospital was effective on ATCC strains (>5log10 reduction) but only moderately effective on VIM microorganisms (median: 1 log10 reduction of the inoculum on the carrier). But alcohol alone (two applications) or the first application with quaternary ammonium and the second with alcohol completely destroyed all microorganisms on the germ-carrier (VIM and ATCC). This destruction was similar to that with the chlorinated product included in this study.

Global incidence of VIM-microorganisms in our hospital was 0.9% (2012) and 0.77% (2013) -non significant differences-. The greatest reduction of incidence was obtained in Neonatology (5.3% in 2012 v.s. 1.9%, in 2013-$p<0.05$-), but in the rest of our hospital (excluding neonates), the VIM-microorganism incidence was 0.6% and 0.7%, respectively in these years (non significant difference).

Figure 1 describes the VIM microorganism colonization or infection tendency by VIM microorganisms. The incidence of colonization was 0.6% in both years but the infection incidence was reduced from 0.3% to 0.1% in 2013. These infections were (relative percentages): respiratory (16.7%), urinary (25%), surgical site (19.4%), septicaemia (22.2%) and "others" (16.7%).

The first thing to point out is that these microorganisms show a very etiological and temporal dispersion (Table 1).There was almost the same number of colonized children each year in the entire hospital, except for neonates (Figure 2), indicating an interesting reduction in new cases, although this figure does not show that there are a large number of prevalent cases (we must multiply by 1.1-1.5 in neonates and by 5-6 in the rest of hospital, giving a prevalence of 2-6% in neonates and 3-4% in other children, that is, an endemic situation). These prevalent cases, in each month, were a mixture of cases from other months and cases already colonized at admission (old cases from our hospital or from others).

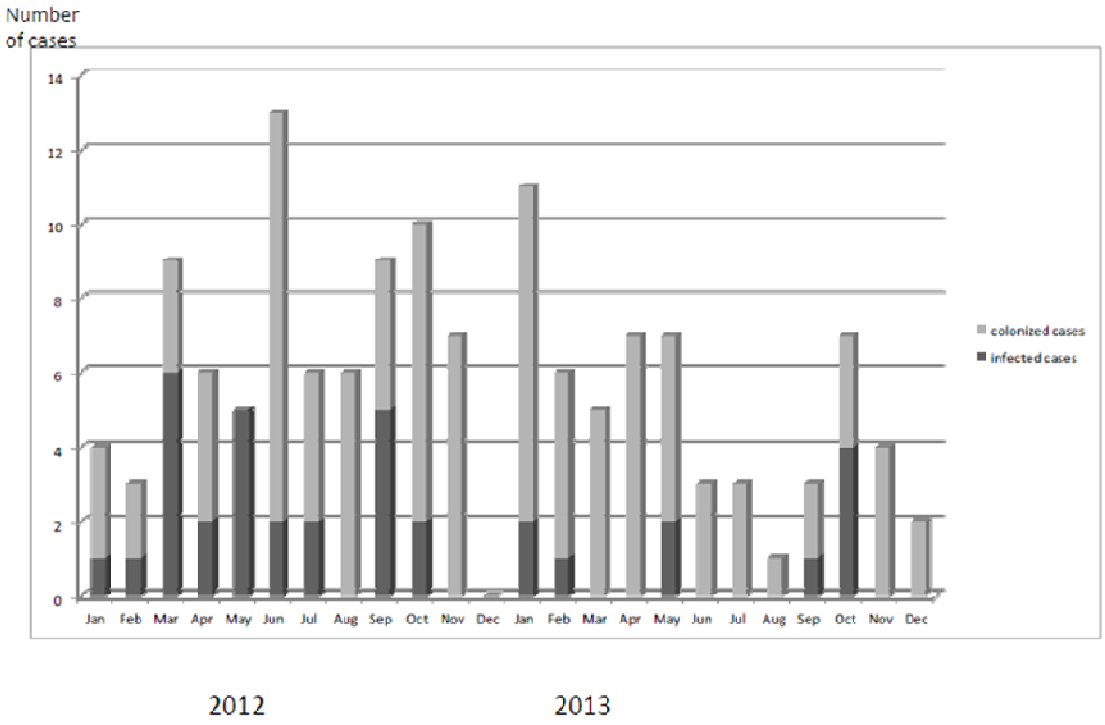

Figure 1: Monthly evolution of infection or colonization number for cases with VIM -microorganisms. Children´s Hospital during two years.

Table 1: distribution of VIM-microorganisms by floor/area in Children´s Hospital, 2012 y 2013 (number of children =137. A child may be colonized by more than one VIM-microorganism)

	Transplant and Oncology	**Neonates**	**Rest of Child**	**Total**
Escherichia coli	7	2	10	19
Klebsiella pneumoniae	17	33	29	79
Klebsiella oxytoca	4	3	5	12
Citrobacter spp	5	2	1	8
Serratia spp	2	1	4	7
Enterobacter spp	12	6	4	22
Pseudomonas spp	13	0	11	24
Other microorganisms	1	1	2	4
Total	61	48	66	175

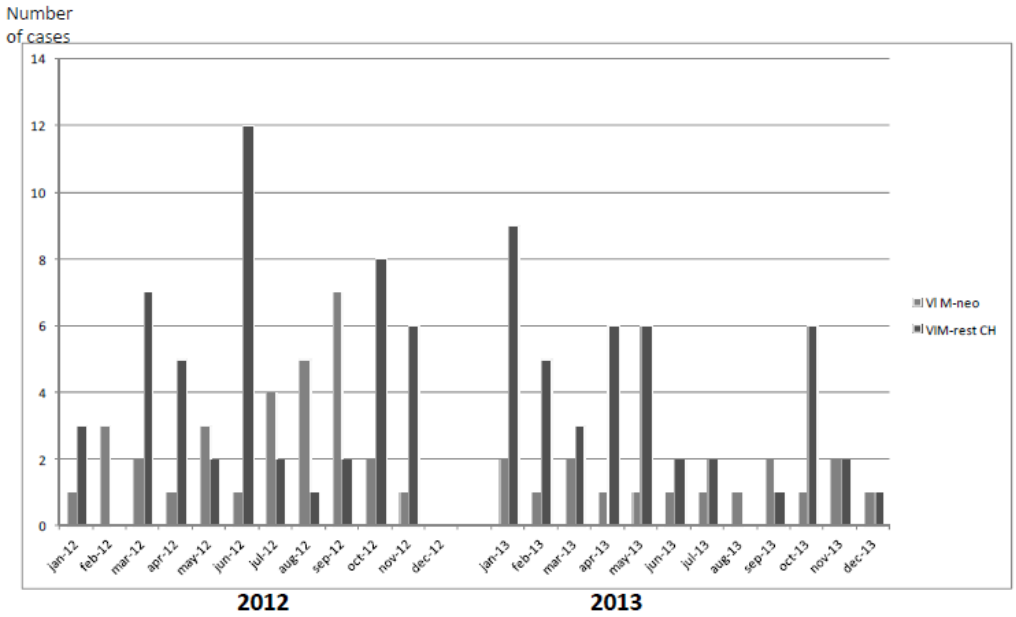

Figure 2: Endemic VIM bacteria**.** Children Hospital La Paz during two years. (monthly trend of new cases from neonates against the rest of this hospital)

Table 2: Characteristics of the 137 patients with VIM-microorganisms in the Children's Hospital stratified by Area of Admission

	TOTAL	**Transplant + Oncology**	**Neonates**	**Rest of children´s Hospital**
	(n=137)	(n=44)	(n=39)	(n=54)
Sex (% male)	48.9	59.1	41	46.3
Age (years, except newborns: in days)				
" Average (SD)	2.7 (5)	4.8 (5.5)	15.5 (39.5) days	2.9 (5.5)
" Median (IQR)	0.6 (0-2.2)	1.9 (0.7-7.8)	0 (0-9) days	0.87 (0.2-2.1)
clinical infection samples (%)	26.3	38.6	10.3	27.8
Previous Admission in our hospital (%)	45.3	63.6	2.6	61.1
Shared floor with another patient with VIM-microorganisms	92	90.9	94.9	90.7
Death during stay (%)	10.2	2.3	10.3	16.7
Time since admission (days)				
" Average (SD)	37.4 (51.9)	35.4 (38.7)	31.8 (27.9)	43 (71.3)
" Median (IQR)	22 (9-48)	20.5 (5.3-52)	23 (10-40)	21 (8.8-45.8)

In cases with VIM there were also several notable facts (Table 2): Most of the 137 cases detected were colonisations (just under 74% of cases, except for neonates, in which 90% were colonisations). The median number of days from admission to diagnosis of VIM microorganisms on our patients was 30-40 days, and mortality during hospital stay (10%, ranging between 2% and 16%), was due to pre-existing health conditions, not the colonisation or infection by VIM microorganisms. Gender distribution was similar (49% male).

The molecular type of some of these microorganisms (cases in some areas and same week) indicated very little cross-transmission if the control measures were taken, as

occurred in Neonates during 2013 vs 2012, reducing the number of VIM bacteria by more than 50% .

DISCUSSION

The most noticeable in the temporal trend over these two years, was the differences between the neonatal area and the rest of the Children's Hospital, where the number of VIM cases tended to rise in 2012. However, the procedures implemented by the HCW during the second half of 2012 decreased the case number, reducing the transmission of VIM (5.3% in 2012 to 1.9% in 2013), despite the ease of transmission in neonates, who present some colonization risk factors, i.e. antibiotic therapy, immature digestive microbiota or deficient immune systems (Martin et al, 2010; Strunk et al, 2011; Sharma et al , 2012; Collado et al, 2012), etc. Moreover we can affirm that this trend was real, without bias, since similar weekly epidemiologic controls of all children admitted were performed during both years, and colonization was detected from the time of birth (or admission from another hospital).

In the rest of the Children's Hospital the control measures were generally not taken for an affected area, but only for the affected child, so the impact or effectiveness was much less, and the trend was stable rather than a decline. We must emphasize that systematic surveillance controls were only done in the PICU and areas with increased infection risk (Transplants and Oncology), and not in others, so there was a detection bias due to the "monitoring effect" (Rothman et al, 2008), with more diagnoses if more controls were performed, as occurred in 2013. Moreover we note recognize that the re-admission of prevalent cases (occurring with more frequency in children other than neonates) can hamper the correct evaluation of the efficacy of these measures.

VIM-microorganisms were of different species (although predominantly *K. pneumoniae*, followed by *P. aeruginosa*, especially in Neonatology and Oncology). They also had different incidences in the different hospital areas. This was probably due to two demonstrated causes:

1) the high environmental resistance of these microorganisms and

2) the ineffectiveness of the quaternary ammonium initially used for surface cleaning.

By mid-2013 we began supplementing surface cleaning with the use of 70° alcohol, since we had seen this also destroyed more than 4 log10 of the VIM-microorganisms. If this double application was not always performed correctly on all surfaces of one room there would be areas where the quaternary ammonium alone was used, and, there would be areas where bacteria were not totally destroyed. We consequently looked for a product that was both an effective cleanser and disinfectant and only required one application.

We are now using a chlorinated compound with 4000 ppm of free Cl^-, with a dual cleansing and disinfection effect (destruction of more than 4 log10 of microorganisms on the same standard surface, where the environmental persistence was assessed). Hopefully the new product, along with a greater discipline in complying with the contact precautions, will reduce the transmission of these VIM-microorganisms and we will achieve a sustained reduction, such as we are have seen during 2014. But this product is not used in Neonatology because it may be an irritant for these more susceptible patients, and the double application of quaternary ammonium and alcohol continues.

IN CONCLUSION

-VIM-Microorganisms were of different species, although predominantly *K. pneumoniae*, followed by *P. aeruginosa*, especially in Neonatology and Oncology.

- We used only antiseptic and surface disinfectants with proved efficacy against to our VIM-microorganisms.

-But VIM-bacteria were not eliminated by the measures taken, and have become endemic to our hospital (principally due to their high prevalence, because the incidence is 0.6-0,7%, except in neonates, 1.9% in the second year).

-Is necessary to increase the detection of new cases in areas without systematic surveillance and to implement correctly all the indicated measures in order to reduce this endemic situation.

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