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MRSA outbreak in a Neonatal Intensive Care Unit in a developed country: importance of rapid detection of reservoirs and implementation of intervention measures

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ABSTRACT

We described a MRSA bloodstream infection outbreak that was rapidly identified and controlled in a Neonatal Intensive Care Unit after implementation of a bundle of measures, including PCR-screening and HCW decolonization. We found 35% of healthcare workers(HCW) colonized with *S. aureus* by PCR, one of them that presented skin lesion positive for MSSA (same clone and *spa* type than two patients). Our findings raise the hypothesis that the outbreak could be related to HCW colonization.

KEYWORDS: *Staphylococcus aureus.* MRSA. Neonatal Intensive Care Unit. Outbreak.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major causative agent of nosocomial infections. In Neonatal Intensive Care Unit (NICU), it can cause serious infection with long sequelae due to immaturity of immunological system, prolonged hospital stay and frequent use of devices by this population[1].

MRSA in NICU may be acquired from colonized parents, healthcare workers (HCW), and other neonates[2]. In Brazil, MRSA is an important causal agent of infection and colonization in NICU[3,4] and the third cause of primary blood stream infection (BSI) in this setting[5]. Several outbreaks have been described in this population, which are frequently prolonged and difficult to control[6,7]. The prompt detection of an outbreak, establishment of infection control measures and identification of possible reservoirs are essential to control and prevent the spread of MRSA in hospital setting[3,4,7,8].

In our institution, the incidence of MRSA infection in NICU was 0.17 infections/1,000 newborns-day in 2015 (two cases in that year), while it was zero in 2016 and 2017 until November. In December 2017, we identified an outbreak due to the occurrence of three cases of bloodstream infection (BSI), which raised the incidence rate for 2.85/1,000 newborns-day in that month. The aim of this study was to describe the investigation and rapid control of MRSA-BSI outbreak in a NICU using two molecular methods.

Ethical approval

Because it was an outbreak and needed rapid response, it wasn't collected

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the personal consent form, however we have the ethical approval for healthcare associated infection investigation. This protocol was approved by the ethical committee from Hospital das Clinicas, from University of Sao Paulo, in Sao Paulo city, Sao Paulo State (N° 4.513.261)

MATERIALS AND METHODS

This is a descriptive analysis of an outbreak investigation in a NICU in Hospital das Clinicas, Sao Paulo, Brazil, a 2,200-bed tertiary university hospital. The NCIU has 48 beds, divided in four physically apart sectors: two areas of intensive care unit with 9 and 16 beds, one intermediate-risk sector with 8 beds and one area with 13 low-risk beds. The unit has around 1,600 admissions per year; 23% of newborns admitted in 2017 were low birthweight (LBW), while 6% were very low birth-weight (VLBW). Each of the sectors has specific physician staff that rotate every 3 months. The nurse: patient ratio in the unit was 1:3 in that period.

Educational training about hand hygiene (HH) is routinely performed every three months for nursing staff. The residents are trained during the internship in NICU. The unit has 30 sinks destinated to hand hygiene and an alcohol-based hand rub available for each bed. During the outbreak, staff educational training regarding hand washing was intensified and awareness about the outbreak occurrence was highlighted.

The 9-bed area represents the sector of higher risk patients, where nurse care is provided only by graduated nurses with Neonatal specialization. In December 2017, we identified an outbreak due to three MRSA-BSI only in this sector. Although the cases occurred in the same area, the newborns were not in adjacent beds.

Immediately after the outbreak recognition, the Infection Control Team (ICT) performed a chart review of newborns. Contact precautions was adopted for MRSA positive children and for children who were inpatient in the 9-bed area until surveillance culture results. Staff hand-washing educational training was intensified. Direct observation of hand hygiene had been performed in June 2017 by ICT and was repeated during January 2018 and April 2018. At these months, manipulation of catheter devices was evaluated as well.

During the first week of January, nasal and axillar swabs were obtained for all children in the 9-bed intensive care unit. Also, nasal swabs were performed for HCW that were involved in assistance during the period from 20th to 31st December 2017. The professionals were also questioned about active skin lesions or soft tissues infections presence. One nurse had pustular lesions and was oriented to be away from assistance until the swab cultures results. For this HCW, we obtained swab samples from her hands as well.

The samples were submitted to aerobic culture specifically for MRSA screening. The identification and antibiotic susceptibilities of the micro-organisms isolated from clinical or swab cultures were determined by Vitek2 system (bioMérieux, Marcy l'Etoile, France), according to Clinical Laboratory Standards Institute Criteria[9].

For culture positive samples, PFGE (Pulsed Field Gel Eletrophoresis) of isolates was performed using a CHEF DR-II (Bio-Rad Laboratories Inc, Grand Junction, CO, USA), as previously described[10]. The profiles were analyzed using Bionumerics version 7.1.6 (Applied-Maths, Sint-Martens-Latem, Belgium). In order to define the Pulsed-Field types (PFT), two cutoff levels were considered: >80 % to define types and 80–95 % to define subtypes. Susceptibility to mupirocin was performed by disk diffusion method and a zone of ≤ 13 mm using a 5µg disk was defined as Mupirocin resistance[11,12].

Additionally, DNA extraction was performed to screening for virulence genes (*luk*E-*luk*D, *pvl* and *tsst*)[7] and clonal complexes using PCR assay[13]. Amplification and sequencing of the SSR region of the *spa* gene were performed with chromosomal DNA purified from each isolate, as previously described[14]. The *spa* type was determined using BioNumerics Bioinformatics software (version 7.1.6., Applied Maths, Sint-Martens-Latem, Belgium). Also, Staphylococcal cassette chromosome *mec* (SCCmec) types I, II, III, IVa, IVb, IVc, IVd and V using specific primers was determined using a multiplex PCR method[15].

After the first week of January, as most surveillance cultures were negative, nasal swabs were collected and MRSA screening was performed by RT-PCR using a commercial kit for SCC*mec-orfX* junction (Xpert® MRSA, Cepheid).

Decolonization using mupirocin ointment and daily bath with clorexidine 2% soap for 7 days was indicated for HCW colonized by MRSA. After decolonization, two nasal swabs were obtained for each professional.

RESULTS

Three cases of MRSA-BSI were identified between 24th December and 31st December 2017. Table 1 describes clinical and epidemiologic aspects of the cases. Two cases had clinical cure and no complicated infection, but one newborn died one day after the positive blood culture. Thus, this child did not collect nasal and axillar swab samples. All children were treated with vancomycin. Cases 1 and 2 demonstrated isolates susceptible to clindamycin,

Table 1 - Clinical and demographic characteristics of 3 newborns identified as having infection by *Staphylococcus aureus resistant to meticilin* in December 2017 in Neonatal Intensive Care Unit of Hospital das Clinicas, Brazil.

Case	Date of blood culture	Lenght of stay until infection (days)	Gestational age (weeks + days)	Birth weight (g)	Vascular device	Days of vascular device	Parenteral Nutrition	Mechanical ventilation	Outcome	Lenght of hospital stay (days)
1	24/12/2017	8	29+3	1230	PICC*	5	Yes	Yes	Discharge	49
2	26/12/2017	7	32+2	2010	Peripheral catether	5	Yes	No	Death	7
3	27/12/2017	6	35+6	1370	PICC	7	Yes	Yes	Discharge	28

*Peripherally inserted Central Catheter.

Table 2 - Results of surveillance cultures and PCR for methicillin-resistant *S. aureus* from nasal swabs from healthcare workers in Neonatal Unit of Hospital das Clinicas, Brazil, January 2018.

Professional category	N	Culture swab collected (%)	Positive swab culture (%)	PCR collected (%)	PCR + S.A. (%)	PCR + MRSA (%)	Discordant results
Nurse	55	20 (36)	0 (0)	39 (71)	13 (33)	4 (10)	4
Physiotherapist	10	5 (50)	0 (0)	5 (50)	2 (40)	1 (20)	1
Senior physician	8	1 (12)	0 (0)	5 (62)	0 (0)	0 (0)	0
Resident physician	12	0 (0)	NA	3 (25)	2 (66)	0 (0)	NA
Resident nurse	5	0 (0)	0 (0)	5 (100)	3 (60)	0 (0)	NA
Total	90	26 (29)	0 (0)	56 (62)	20 (36)	5 (9)	13

PCR + S.A = positive PCR for *S. aureus*; PCR + MRSA = positive PCR for MRSA; Discordant results = number of people who collected both methods (culture and PCR) with discordant results.

ciprofloxacin and cotrimoxazole. Case 3's isolate demonstrated resistance to clindamycin, ciprofloxacin and was susceptible to cotrimoxazol.

Nasal and axillar swab samples were obtained from 9 newborns. Except for the two newborns with known infection, no children were positive for *S. aureus* by cultures or PCR detection.

We identified 90 HCW involved in assistance from 20th to 31st of December. Twenty-six professionals collected nasal swabs for aerobic culture while 56 collected swabs for RT-PCR. Twelve HCW were screened by both methods. Twenty HCW did not collected samples because were on vacations, in other rotations or working in other sectors during investigation period. There were no HCW positive for MRSA when screened by aerobic culture. The HCW that had skin lesions during the outbreak period had hand's culture positive for methicillin-susceptible *S. aureus*. She was allowed to work after complete recovery of the lesions.

Results of PFGE, *spa* typing and SCCmec amplification for culture positive isolates are demonstrated in Figure 1. Case 1 and 2 demonstrated similarity with the HCW strain, while case 3 was a different clone. All MRSA isolates were susceptible to mupirocin, positive for gene LuKDE and for Complex clonal 5. Panton Valentine Leucocidin (PVL) and TSST-1 were absent for all isolates. Results of PFGE were available after two weeks of screening. All strains were susceptible to mupirocin.

Five HCW were positive for MRSA by PCR method. These professionals performed decolonization and collected new samples between 12 and 70 days after the procedure.

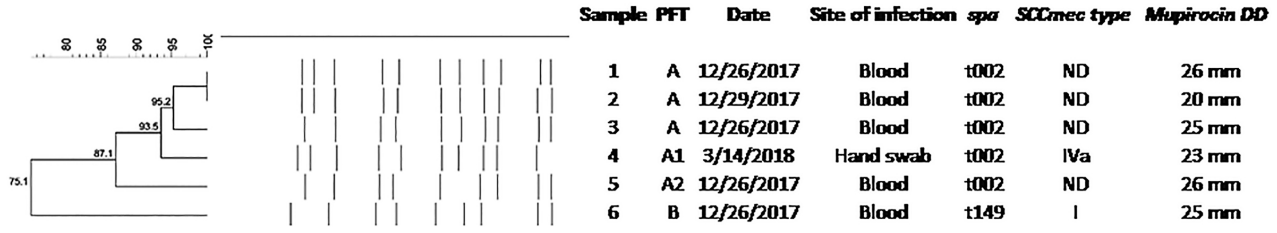

Figure 1 - Dendogram and spa type analysis of three cases of *S. aureus* isolated in blood samples from newborns and from healthcare worker (HCW). PFT = Pulsed Field Type; ND = not determined; Mupirocin DD = mupirocin DD test.

One physiotherapist persisted colonized with MRSA in the follow-up sample, but decolonization procedure was not repeated. Among HCW colonized by MRSA, three were identified as having *spa* type 002 and one was positive for *spa* type 127. There was no amplification for one of the five positive swab samples. These samples were negative for all SCCmec tested in this study. Results of *spa* typing were available after 72h of RT-PCR screening.

Hand hygiene compliance was 63% in June 2017, raised to 74% during January 2018 and to 84% in April 2018, after training and orientation of HCW. Hand hygiene Compliance before catheter manipulation was poor (50%) in January 2018. After training focused on catheter manipulation, compliance for this moment increased for 82% in April 2018.

DISCUSSION

In neonatal setting, MRSA infection is associated with increased morbidity, prolonged hospitalization, and excess of cost[1]. In our study, all children had low birth weight and received parenteral feed which demonstrate the occurrence in patients with severe underlying illness. Importantly, the infection was detected with a mean of 7 days after birth, indicating early pathogen acquisition.

Considering that acquisition by contact of adult skin and horizontal spread are described as important forms of acquisition of MRSA in NICU and occurrence of three cases in one week, we hypothesized that the outbreak could be related to colonization of HCW and/or pathogen cross-transmission. We found 35% of HCW colonized by *S. aureus* by PCR, one of them that presented skin lesions. Since *S. aureus* colonization varies in adult population, we prioritize decolonization of the employee with skin lesions and for HCW who were MRSA positive, which represented 8.9% of the HCW screened. This is a high rate of colonization when compared with other studies[6]. The use of PCR method was more effective to detect colonized HCW and implement decolonization procedures. Although most dispendious, the sensitivity and rapid result of this method should be considered during an outbreak investigation to allow fast implementation of control measures[16].

Two children had infection by non-multidrug-resistant MRSA, whose isolates remained susceptible to clindamycin, ciprofloxacin and cotrimoxazole. Garcia *et al*.[2] previously demonstrated an increase prevalence of this susceptibility profile in newborns in our service and that was probably related to nosocomial transmission instead of maternal transmission. These strains cared virulence factors which could explain the severity of infection progression in Neonate 2. Unfortunately, it was not possible to determine Sccmec types for these isolates by multiplex PCR.

Although the almost concomitant occurrence, Neonate 3 isolated a different clone, which indicates more than one source of transmission. We could not clarify if this child had infection acquired by a HCW not screened during the investigation.

The use of *spa* SSR region sequencing may be more rapid and convenient for outbreak investigation in the hospital setting since *spa* typing involves a single locus, and PFGE is a laborious and time consuming technique. This tool has been used for outbreak investigation as well as to investigate routs of *S. aureus* transmission[17-19]. The type t002 founded in most HCW has been frequently described in neonates and related to outbreaks in France[8], while t149 has been described as cause of skin and soft tissue infections in Brazil and other Latin America countries[20]. In this study, spa typing and PFGE analysis leaded to similar conclusions about isolates' similarities and suggested MRSA acquisition by HCW. Thus, in a low resource scenario, *spa* typing seems to be a more effective method to rapidly investigate and control outbreaks.

CONCLUSION

We described a MRSA-BSI outbreak in a NICU that was prompted investigated and controlled after HCW decolonization and reinforcement of hand hygiene and catheter-care. The use of *spa* SSR region sequencing may be more rapid and convenient for outbreak investigation than PFGE that is a laborious and time-consuming technique.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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