



Case study

Polymerase chain reaction-based open reading frame typing (POT) method analysis for a methicillin-resistant *Staphylococcus aureus* (MRSA) outbreak through breast-feeding in the neonatal intensive care unit



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ABSTRACT

Introduction: The route of methicillin-resistant *Staphylococcus aureus* (MRSA) transmission in the neonatal intensive care unit (NICU) is not clearly explained. We investigate an MRSA outbreak involving five babies in the NICU. The molecular investigation using polymerase chain reaction-based open reading frame typing (POT) method was performed.

Presentation of outbreak: A MRSA outbreak occurred in a six-bed NICU affecting 5 babies. Within 13 days of the emergence of index case, all five babies including triplets and other two babies were found to colonize MRSA by the active surveillance culture. Environmental surveillance cultures revealed that the preserved breast milk provided by the triplets' mother was the only item in the NICU that was positive for MRSA. The mother had a bite wound on the nipples, and the breast milk was not pasteurized. The POT method revealed that MRSA strains detected from the triplets, the breast milk, and the other baby who was fed the triplets' mother's milk were genetically identical (POT index: 106-247-33). The all strains of MRSA carried Staphylococcal cassette chromosome *mec* (SCC*mec*) IV and had good susceptibility for the non-β-lactam antimicrobial agents, suggesting the strains were community-acquired MRSA.

Conclusions: The mother's milk contaminated with community-origin MRSA is serving as the reservoir of MRSA and one of the sources of MRSA outbreaks in the NICU. It is important to closely monitor the condition of the mothers of the children in the NICU. Pasteurization of breast milk should be considered when the skin on the nipple is broken.

Introduction

Neonates, particularly those with a very low birth-weight, are particularly susceptible to infection by environmental microbes such as *Staphylococcus* species [1]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a virulent bacterium in the neonatal intensive care setting, and colonization by MRSA is associated with increased morbidity, which places a substantial economic burden on hospitals [2]. In addition, the neonate intensive care setting is often crowded with staff, patients' family members, and equipment, making it difficult to control bacterial transmission [3]. The route of methicillin-resistant

Staphylococcus aureus (MRSA) transmission in the neonatal intensive care setting is often not clearly explained. Here we describe an outbreak of MRSA colonization in five neonates admitted to the six-bed neonatal intensive care unit (NICU) of a local tertiary general hospital in Japan in November 2016. We performed the epidemiological and molecular genetic analysis using polymerase chain reaction-based open reading frame typing (POT) method to identify the route of MRSA transmissions.

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Description of the outbreak

In our institution, NICU contains six open-floor beds and is staffed by pediatric specialists and dedicated nursing staff. Approximately 160 neonates are admitted to the NICU every year. Neonates are screened by means of bacterial culture from the nose, skin, and feces upon admission and weekly nasal screening, and screening of other specimens anytime as well if applicable. In our institution, this active surveillance has detected one or two cases of MRSA colonization per year without a major outbreak.

On November of 2016, triplets born at a neighborhood university hospital were transferred to our hospital. At the time of transfer, two of the triplets (neonates A and B) were reported as being colonized with MRSA in the previous hospital. The remaining triplet (neonate C) was later found to be colonized with MRSA through the active surveillance conducted in this NICU. The mother of the triplets reported having a wound on her nipple by sucking of her babies. In our hospital, the mother used a breast pump to express milk that was then stored in the refrigerator without pasteurization. One day, breast milk expressed by the mother of the triplets was accidentally given to one of the other neonates in the NICU (neonate D). In this NICU, pasteurization of breast milk intended for a mother's own child is not a routine procedure. Active culture surveillance conducted on the next week revealed that neonates D and E were also colonized with MRSA. Because all five neonates were positive for MRSA colonization, the hospital's infection control committee considered the situation was an MRSA outbreak and closed the NICU to new admissions. To identify the route of transmission of the MRSA, environmental screening was conducted of the surfaces in the NICU and the breast milk accidentally fed to neonate D. The environment screening did not reveal MRSA colonization of any of the tested surfaces in the NICU, including the surfaces of items that are frequently handled. The stored breast milk was only positive for MRSA. The isolated MRSA from neonates A–C (triplets), D, and E, and from the contaminated breast milk were all found to be susceptible to the non- β -lactam agents gentamicin (minimum inhibitory concentration [MIC] $\leq 1 \mu\text{g}/\text{mL}$), minocycline (MIC $\leq 2 \mu\text{g}/\text{mL}$), and sulfamethoxazole/trimethoprim (MIC $\leq 1 \mu\text{g}/\text{mL}$), but resistant to levofloxacin (MIC $> 4 \mu\text{g}/\text{mL}$). A polymerase chain reaction-based open reading frame typing (POT) method (CicaGeneus Staph POT KIT, Kanto Chemicals, Tokyo) was used to determine whether the isolated MRSA were the same or different strains. The POT index (POT1-POT2-POT3) was determined by the bands on the electrophoresis gel according to the manufacturer's instruction as appropriate and strains with identical POT index were considered to be the same strain. The MRSA isolated from neonates A to D and the breast milk were considered to be the same strain (POT index, 106-247-33) (Fig. 1). The MRSA isolated from neonate E was considered to be a different strain (POT index, 106-183-32), suggesting the strain from the neonates E was different origin. Staphylococcal cassette chromosome *mec* (SCC*mec*) typing was also performed as previously reported [4]. The isolated MRSA were all found to carry SCC*mec* type IV.

Additional infection control measures including education of the NICU staff and encouraging hand hygiene and appropriate use of personal protection equipment before and after the handling of breast milk were also implemented. No neonates developed MRSA infection. By two month later, all five neonates were successfully discharged without developing MRSA infection and no other cases of MRSA colonization were detected.

Discussion

The results of the present investigation suggested that mother's milk was the reservoir of MRSA that had characteristics of community-acquired MRSA [5]. The routes of transmission of MRSA in the NICU setting vary: colonized healthcare workers [6], an over-crowded NICU environment [7], and mother-child contact [8]. An MRSA outbreak

involving a mother who developed mastitis and contaminated breast milk has also been reported [9,10].

Breast milk is important for the development of the barrier function of the mucosal barrier in neonates, and administering colostrum to neonates is beneficial in the NICU setting for preventing necrotizing colitis and bacterial infection [11]. However, the handling of colostrum or breast milk requires strict adherence to procedures describing hand hygiene and the cleaning of milking and storage equipment [12]. Soreness of the nipples is an underlying condition for colonization of the nipple by bacteria, especially *Staphylococcus* species, or for the development of mastitis [13]. Low-temperature pasteurization of donor milk such as Holder method (62.5 °C for 30 min) is recommended [14], although low-temperature pasteurization decreases the efficacy of endogenous anti-septic compounds in milk [15] and is not commonly used for milk intended for the mother's child. Another report from Japan describes the transmission of *Escherichia coli* via shared breast milk collected from a mother whose nipple was wounded and was not disinfected prior to milking [16]. Improving nipple pain has shown that the use of nipple protection devices such as breast shells may decrease the incidence of nipple trauma and pain from bite wounds [17]. Thus, it remains important to closely monitor the condition of the mothers of the children in the NICU.

Community-acquired MRSA strains carrying SCC*mec* IV are currently the dominant strains in NICU transmission. Carey et al. analyzed MRSA strains isolated from NICUs in the United States and reported that the dominant SCC*mec* type had changed from type II in 2000 to type IV in 2005 [18]. Our previous study also suggested that type IV strains were the dominant MRSA strains in the NICU of university hospital in Japan [19]. We consider active surveillance and isolation of colonized or infected patients to still be viable means of controlling MRSA transmission in the NICU. The POT method is reported to be as equally efficacious as pulsed-field gel electrophoresis analysis for the identification of MRSA strains [20], and it only requires 4 to 6 h, compared with the pulse-field gel analysis that requires 20 h and more. In addition, a number of 106 in POT1 were associated with MRSA carrying SCC*mec* IV [20]. Furthermore, to prevent breast milk mid-administration, procedures such as a double check at the time of pumping and administration or implementing a breast milk barcode scanning system will be needed from the viewpoint of medical safety [21].

Conclusions

We investigated an outbreak of MRSA colonization involving five neonates in the NICU. Based on epidemiological and molecular genetic typing by using a POT method, the MRSA outbreak was suspected to have originated from the contaminated breast milk. The causative MRSA strain had the characteristics of community-acquired MRSA. Care of wounded nipples and the pasteurization of breast milk prevent future MRSA transmission in the NICU.

Compliance with ethics guidelines

This study was approved by the ethics committee of Yokohama Medical Center (approval no. 28-31).

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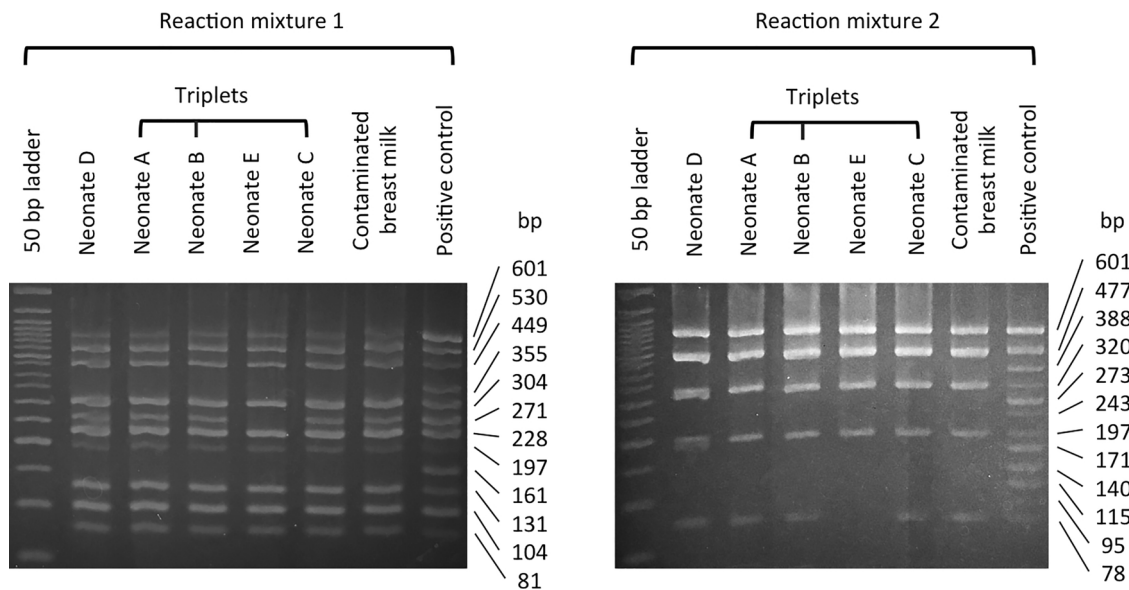


Fig. 1. A polymerase chain reaction-based open reading frame typing (POT) method analysis of the MRSA from the triplets, two other babies and the breast milk.

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