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Comparison of USA300 with non-USA300 methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit

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ABSTRACT

Objectives: Reports of USA300 methicillin-resistant *Staphylococcus aureus* (MRSA) strain were still scarce in neonatal intensive care units (NICUs) and the relationship of USA300 MRSA to clinical infections is still controversial. The primary outcome was the incidence of MRSA infections caused by the USA300 and non-USA300 strains at a NICU in Japan.

Methods: This retrospective cohort study was conducted between November 2011 and October 2016 at Tokyo Metropolitan Children's Medical Center in Japan. All MRSA isolated after 48 h of hospitalization were included for analysis by pulsed-field gel electrophoresis (PFGE) using the standard USA300 strain. Genes were tested for Panton-Valentine leukocidin (PVL) and arginine catabolic mobile element (ACME). A whole genome sequence was performed for representative isolates of USA300.

Results: In total, 109 MRSA isolates were included for analysis. PFGE classified 34 and 75 isolates of USA300 and non-USA300 MRSA, respectively. Both PVL and ACME genes were detected in USA300 and non-USA300 strains at rate of 100% (34/34) and 5.3% (4/75), respectively ($P < 0.05$). There was no statistically significant difference in the proportion of clinical diseases between USA- 300 and non-USA 300 strains.

Conclusions: Infants with USA300 MRSA infection did not differ significantly from those with non-USA300 MRSA infection.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been a major cause of nosocomial infection since the 1980s. The first MRSA infection treated in a neonatal intensive care unit (NICU) was reported from the United States (US) in 1981 (Weeks et al., 1981). Neonates are particularly vulnerable to invasive MRSA infections due to having multiple risk factors including immature immunity, skin prematurity, long-term hospitalization, and frequent uses of medical devices.

The prevalence of specific MRSA strains varies by region. One of the MRSA strains circulating in the US is the USA300 strain, which was initially reported in the community (Moran et al., 2006). Recently, the USA300 strain was reported to have spread from the community to healthcare facilities including NICUs (Diekema et al., 2014; Healy et al., 2004). The first USA300 MRSA infection in Japan was reported as an imported case in a 3- month old girl born in the US in 2008 (Shibuya et al., 2008). The USA300 strain has been detected in Europe, Latin America, the West Pacific, and the Caribbean (Nimmo, 2012; van der Mee-Marquet et al., 2015). In Japan, two small outbreaks at hospitals were reported, with both cases involving adult patients and healthcare workers (Nagao et al., 2010; Mine et al., 2011). Community-

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acquired recurrent skin infections in children were also reported in families (Uehara et al., 2015). In the largest report, 1.2% (42/3433) of MRSA isolated from patients at nine hospitals in Tokyo consisted of the USA300 strain (Takadama et al., 2018). Although the USA 300 strain is still rare in Japan, close monitoring of epidemiology is warranted.

The USA300 strain was originally identified by its pulsed-field gel electrophoresis (PFGE) pattern (McDougal et al., 2003). The genotype belongs to sequence type 8 (ST8) and staphylococcal chromosomal cassette *mec* (SCC*mec*) type IVa. The pathogenicity of the USA300 strain was linked to carriage of Panton–Valentine leukocidin (PVL) and the arginine catabolic mobile element (ACME) (Diep et al., 2006). PVL is a toxin capable of lysing white blood cells. However, the clinical significance of PVL in relation to the development of severe diseases is still controversial (Shallcross et al., 2013). ACME is associated with an adaptation to acidic environments such as human skin via the inhibition of nitric acid production (Gordon and Lowy, 2008).

In terms of virulence, the presence of the USA300 clone among community-acquired methicillin-susceptible *S. aureus* resulted in a higher risk of developing osteomyelitis than the presence of a non-USA300 clone in children (McCaskill et al., 2007). In contrast, USA300 MRSA infections reported in the US were not associated with higher mortality than non-USA300 MRSA infections according to a systematic review (Nair et al., 2014). However, there were no reports comparing the clinical features and outcomes between USA 300 and non-USA 300 strains in the NICU. The aim of our study was to compare the incidence of USA300 MRSA infections and non-USA300 MRSA infections in the NICU.

Methods

Patient selection

This retrospective cohort study was conducted between November 2011 and October 2016 at Tokyo Metropolitan Children's Medical Center in Japan. Our children's hospital is equipped with 24 beds in the level 3 NICU and 48 beds in the level 2 NICU.

All MRSA isolated from patients in the NICU after 48 h of hospitalization were included. If multiple MRSA strains were isolated from same patient at different times, the first isolate causing infection or colonization was included. If colonization occurred prior to infection, the first isolate causing infection was analyzed. Infection was defined as a clinical disease requiring a full-course of anti-MRSA antimicrobial treatment. Colonization was defined as the detection of MRSA from non-sterile sites in the absence of clinical symptoms which does not require specific treatment besides decolonization. Sterile sites were defined as blood, cerebrospinal fluid, peritoneal fluid pleural fluid and specimen obtained from surgery or aspirate from internal body sites. Active surveillance for MRSA was conducted biweekly using nasal swabs in MRSA- negative patients during the study period. The isolates detected from active surveillance were also included for this study.

Patient's data including sex, age, birth weight, gestational age, Apgar score, and length of hospital stay were collected from the medical records.

Laboratory methods

Identification and susceptibility testing for MRSA were conducted in accordance with the Clinical Laboratory Standards Institute (CLSI) document M100-S25 (Patel et al., 2015) with MicroScan WalkAway® 96 plus System (Beckman Coulter Inc., Brea, CA, the US).

Molecular typing

Polymerase Chain Reaction for PVL and ACME

DNA from the MRSA isolates was extracted using QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) in accordance with the manufacturer's instructions. PCR was performed to detect the presence of the genes encoding Panton-Valentine leukocidin (PVL) and arginine catabolic mobile element (ACME) (Goering et al., 2007; Ma et al., 2008).

Pulsed-field gel electrophoresis

All isolates were analyzed by pulsed-field gel electrophoresis (PFGE). Crude DNA of the strains was digested with *SmaI* and electrophoresed by CHEF-MAPPER (Bio-Rad, Hercules, the USA). USA300-0114 MRSA was used as the reference.

Whole genome sequencing (WGS)

Whole genome sequencing (WGS) was performed using DNA of the strains with indistinguishable PFGE band pattern from USA300-0114. Nextera XT DNA sample preparation kit (Illumina Inc. California, USA) was used for sample preparation, and Illumina MiSeq platform (Illumina Inc. California, USA) was used for sequencing, followed by de novo assembly using CLC Genomics Workbench version 9 (Qiagen N.V. Venlo, Netherlands).

Multilocus sequence typing (MLST)

The assembled WGS contigs of were submitted to MLST 1.8 on the Center for Genomic Epidemiology (CGE) website (Larsen et al., 2012) to identify the multilocus sequence types (STs).

*SCC*mec *typing*

SCC*mec* typing was performed using WGS and SCC*mec* finder 1.2 on the CGE website, along with the multiplex PCR methods (Kondo et al., 2007; Larsen et al., 2012).

Definition of USA300

The strains with indistinguishable PFGE band pattern with USA300-0114, ST8, SCC*mec* IV, PVL and ACME genes were defined as USA300 in this study.

Outcomes

The primary endpoint was the proportion of MRSA infections caused by USA300 and non-USA300 MRSA strains.

Statistical analyses

We performed descriptive statistics using medians and interquartile ranges for continuous data and percentages for categorical data. Categorical variables were compared using the chi-squared test or Fisher's exact test if the expected values were below 10. All of the statistical analyses were performed using SPSS 24 (IBM SPSS, Chicago, IL, USA). All of the tests were two-tailed and $p < 0.05$ was considered statistically significant.

Results

Of the 130 MRSA isolates, 109 (84%) were included for analysis. Twelve isolates with duplication and nine isolates detected within 48 h of hospitalization were excluded. All strains were susceptible to vancomycin and linezolid. Susceptibility of the USA300 and non-USA 300 strains to clindamycin was 100% (34/34) and 35% (26 of 74), respectively (Table 1).

PFGE-classified USA300 and non-USA300 MRSA comprised 34 isolates (31.2%) and 75 isolates (68.8%), respectively. USA300 isolates were also confirmed as ST8 and carrying SCC*mec* IVa. Both

Table 1
Antimicrobial susceptibility results for methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit.

	USA 300 (n = 34)	Non-USA300[a] (n = 74)
Vancomycin (%)	34 (100)	74 (100)
Linezolid (%)	34 (100)	74 (100)
Gentamicin (%)	33 (97)	56 (75.6)
Clindamycin (%)	34 (100)	26 (35.1)

[a] One case of non USA300 had no data on antimicrobial susceptibility.

the PVL and ACME genes in the USA300 and non-USA300 strains were detected at a rate of 100% (34/34) and 5.3% (7/75), respectively. The demographic characteristics of the USA300 and non-USA300 strains are shown in Table 2. There was no significant difference besides the detection of the PVL and ACME genes. Four patients colonized with MRSA subsequently developed infection. However, all of the initial colonized MRSA strains were different from MRSA strains causing subsequent infections by PFGE analysis.

Although the proportion of clinical infections was as high as 40% for USA300 MRSA, there was no statistically significant difference from the non-USA 300 strains (Table 3). Two deaths within 30 days due to lower respiratory infection and bacteremia were observed among the patients infected with a non-USA 300 strain. Our study did not show the recurrence of soft tissue infections (SSTI) in both groups.

Neonatal toxic shock syndrome-like exanthematous disease (NTED) is generally seen in infants colonizing *S. aureus* producing the toxic shock-syndrome toxin 1 (Takahashi et al., 1998). NTED usually appears in the first few days of life with fever and a widespread macular erythema. Thrombocytopenia and a mild increase of C-reactive protein are also present. The disease is less severe than toxic shock syndrome and self-limiting.

Table 3
Comparison of the characteristics of USA 300 and non-USA 300 methicillin-resistant *Staphylococcus aureus* detected cases in a neonatal intensive care unit.

	USA 300 n = 34 (%)	Non-USA300 n = 75 (%)	*P* value
Clinical infections	14 (41.2)	26 (34.7)	0.5274
Bacteremia	7 (20.6)	8 (10.7)	0.229
All SSTIs	6 (17.6)	10 (13.3)	0.568
Subcutaneous abscess	4 (11.8)	3 (4)	0.201
Liver abscess	1	0	0.312
NTED	0 (0)	4 (5.3)	0.307
Osteomyelitis	1 (7.1)	2 (2.6)	1
LRTI	0	3	0.551
Unkown origin[a]	3	3	0.373
Colonization	20 (58.8)	49 (65.3)	0.229
Mortality cases	0	2	1

SSTIs, skin and soft tissue infections; NTED, Neonatal toxic shock syndrome-like exanthematous disease; LRTI, lower respiratory tract infections.
[a] Cases in which methicillin-resistant *Staphylococcus aureus* was detected from a non-sterile site such as the nasal mucosa, and the clinician decided the infectious disease by clinical presentation, and the treatment was completed.

Discussion

This study found no significant difference between the USA300 and non-USA300 strains in the proportion of MRSA clinical infections in an NICU. MRSA infections may cause a significant burden on infants in a NICU that the mortality rate reported by a previous study was 11.9% (Ericson et al., 2015), while the rate found in our study was 5% (2/40) (Table 4).

Nonetheless, whether the USA300 strains are associated with higher mortality remains controversial. In a study of MRSA bloodstream infections mainly among adults, USA300 was associated with higher seven-day mortality (Kempker et al., 2010). However, a number of studies failed to confirm this association (Nair et al., 2014; Kempker et al., 2010; Simor et al.,

Table 2
Comparison of the characteristics of USA 300 and non-USA 300 methicillin-resistant *Staphylococcus aureus* detected cases in a neonatal intensive care unit.

	USA 300 (n = 34)	Non-USA 300 (n = 75)	*P* value
Male sex (%)	18/34 (53)	40/75 (53)	
Age, days, median (IQR)	17 (10–22)	22 (8–39)	0.446
Birth weight, g, median (IQR)	1787.5 (1385–2555.5)	1915 (885–2660)	0.395
<1000g	6	23	
1000 to <1500g	4	9	
1500 to <2500g	14	18	
>−2500g	10	25	
Gestational age, wks, median (IQR)	32.5 (29.5–37)	33 (27–38)	0.725
<28 wks	4	22	
28 to <32 wks	7	12	
32 to <37 wks	12	9	
37 to <42 wks	11	32	
Mode of delivery			
Cesarean section (%)	23/34 (68)	49/75 (65)	1
Apgar score at 1 min, score, median (IQR)[a]	7 (3–8)	6 (4–9)	0.374
Apgar score at 5 min, score, median (IQR)[b]	8 (5–9)	8 (7–9)	0.887
Hospital stay, days, median (IQR)	67.5 (37.5–123.5)	74 (34.75–134.5)	0.576
PVL(%)	34/34 (100)	4/75 (5.3)	<0.001
ACME (%)	34/34 (100)	4/75 (5.3)	<0.001

IQR, interquartile range; PVL, Panton-Valentine leukocidin; ACME, arginine catabolic mobile element
[a] One case of USA300 and four cases of non-USA300 had no record of the Apgar score at 1 min.
[b] One case of USA300 and seven cases of non-USA300 had no record of the Apgar score at 5 min.

Table 4
The sites where methicillin-resistant *Staphylococcus aureus* was detected.

	Clinical infections	Colonization	Total
Nasal swab	6	59	65
Stool	17	4	21
Blood	15	0	15
Sputum	10	5	15
Throat swab	9	2	11
Subcutaneous abscess	7	2	9
Umbilical region	7	2	9
Catheters	5	0	5
Urine	3	0	3
Skin swab	1	2	3
Wound area	2	0	2
Eye mucus	1	1	2
Bedsore	1	0	1
Total	84	77	161

Samples were included from the same patients at the same time.

2016; Gasch et al., 2013; Tattevin et al., 2012; Wang et al., 2010). Our study also failed to find any significant association of a higher proportion of clinical diseases and mortality with USA300 strains in infants vulnerable to MRSA infection. Recurrent skin and SSTI due to vertically transmitted USA300 was reported in a NICU (Nurjadi et al., 2017). However, our study did not find any significant difference in the proportion of SSTI infections between USA300 and non-USA300 MRSA strains.

We detected the first case with the USA300 MRSA from a patient who stayed at our NICU for a prolonged period of time in 2014. Since then, USA300 strains were isolated sporadically. MRSA transmission is caused by contact with reservoirs who could be patients, health care workers or even contaminated environments. The reason for spread of USA300 strains in our NICU was not clear. Our speculation was that the USA300 strain was introduced by chance in our NICU and spread by contact.

Our study has several limitations. First, this study was conducted at a single center with a limited sample size. However, this is the first study of the clinical severity of USA300 MRSA infections focused on infants in an NICU. Second, we did not perform WGS for all MRSA strains. However, PFGE and the detection of the PVL and ACME genes were adequate for accurate identification of USA300. Third, active surveillance was used during outbreaks to identify additional cases. Active surveillance was implemented only 4 times during 5 years. Each active surveillance was carried out for several weeks. Outbreaks caused by USA300 and Non-USA300 were once and thrice, respectively. Influence of active surveillance was not substantial. The number of colonizations might be underestimated without active surveillance.

In conclusion, there was no significant difference in the proportion of MRSA clinical diseases between infants with an USA300 MRSA infection and those with a non-USA300 MRSA infection.

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Ethical approval

Permission to carry out this study was granted by The institutional review board of Tokyo Metropolitan Children's Medical Center (H28b-161).

Conflict of interest

The authors would like to declare no potential conflict of interest.

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