



## Note

## First report on USA300 outbreak in a neonatal intensive care unit detected by polymerase chain reaction-based open reading frame typing in Japan

Yuki Uehara <sup>a, b, \*</sup>, Miki Mori <sup>a</sup>, Mitsuhiro Tauchi <sup>a</sup>, Shu Nishimura <sup>a</sup>, Hiroki Sakurai <sup>c</sup>, Takemi Murai <sup>c</sup>, Kaoru Okazaki <sup>d</sup>, Kazue Kinoshita <sup>e</sup>, Yuho Horikoshi <sup>c</sup>, Keiichi Hiramatsu <sup>a, f</sup>

<sup>a</sup> Department of Infection Control Science, Juntendo University Graduate School of Medicine, 2-1-1, Hongo, Bunkyo-ku, Tokyo 113-8421, Japan

<sup>b</sup> Department of General Medicine, Juntendo University Faculty of Medicine, 2-1-1, Hongo, Bunkyo-ku, Tokyo 113-8421, Japan

<sup>c</sup> Division of Infectious Diseases, Department of Pediatrics, Tokyo Metropolitan Children's Medical Center, 2-8-29, Musashidai, Fuchu, Tokyo 183-8561, Japan

<sup>d</sup> Division of Neonatology, Tokyo Metropolitan Children's Medical Center, 2-8-29, Musashidai, Fuchu, Tokyo 183-8561, Japan

<sup>e</sup> Division of Molecular Laboratory, Tokyo Metropolitan Children's Medical Center, 2-8-29, Musashidai, Fuchu, Tokyo 183-8561, Japan

<sup>f</sup> Infection Control Science Research Center, Juntendo University Graduate School of Medicine, 2-1-1, Hongo, Bunkyo-ku, Tokyo 113-8421, Japan



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## ABSTRACT

Outbreaks of methicillin-resistant *Staphylococcus aureus* (MRSA) in the neonatal intensive care unit (NICU) have been reported worldwide. Some outbreaks were caused by USA300, which is a community-associated MRSA clone. In 2011, polymerase chain reaction-based open reading frame typing (POT) for the initial MRSA isolates from all inpatients was started at the Tokyo Metropolitan Children's Medical Center. From March 2014 to April 2015, a total of 131 MRSA strains were isolated, 104 of which were analyzed as healthcare-associated MRSA. Thirteen stains (12.5%) had a POT number of 106-9-93, which strongly suggested USA300; these included 6 from nasal swabs, 6 from blood cultures and 1 from subcutaneous pus. All the MRSA strains were isolated from patients in the NICU; were typed as sequence type 8, *spa* type t008, and staphylococcal cassette chromosome type *mec* IVa; and possessed the *lukS-lukF* and arginine catabolic mobile element-*arcA* gene. Pulsed-field gel electrophoresis of all the strains, with USA300-0114 as a reference, showed indistinguishable banding pattern. Based on these results, POT was useful in recognizing this first MRSA outbreak of USA300 in a Japanese NICU and was advantageous in terms of swiftness, less cost and monitoring change of the epidemic MRSA lineage.

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Outbreaks of methicillin-resistant staphylococcus aureus (MRSA) in neonatal intensive care units (NICUs) have been reported in many countries [1,2]. In the United States, the clone USA300, which was originally recognized as a representative community-associated MRSA (CA-MRSA) in 2000s, has been reported as the healthcare-associated MRSA (HA-MRSA) clones in the NICU [3,4]. Moreover, USA300 has been known to spread rapidly worldwide and to cause severe infectious diseases, such as skin and soft tissue infections (SSTIs) and pneumonia [5,6]. Recently, USA300 was

reported to be sporadically isolated in several Japanese hospitals, and a nosocomial outbreak of USA300 was also reported from Okinawa, Japan [7,8]. Based on these facts, special attention on the detection of USA300 in Japanese NICUs is necessary.

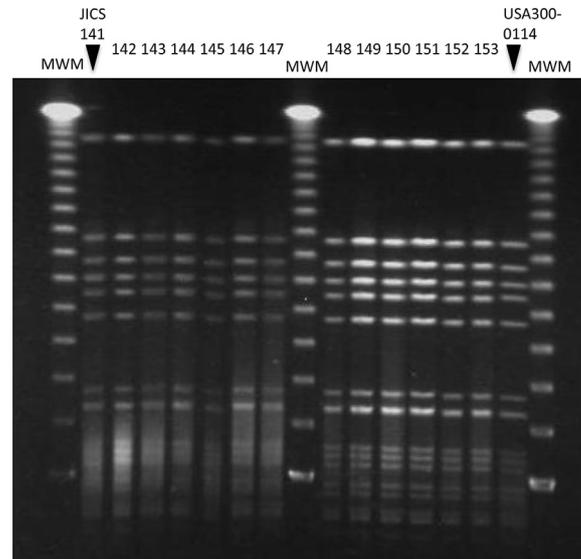
The Tokyo Metropolitan Children's Medical Center is one of the largest tertiary children's hospitals in Japan, with 72 beds in the NICU. In 2011, polymerase chain reaction (PCR)-based open reading frame typing (POT) for the initial MRSA isolates from all inpatients was started in the hospital as a convenient multiplex PCR tool to estimate the molecular relatedness of MRSA [9]. In this article, we reported that POT enabled successful detection of a USA300 outbreak in the NICU, and the usefulness of POT for monitoring USA300 transmission was evaluated.

\* Corresponding author. Department of Infection Control Science, Juntendo University Faculty of Medicine, 2-1-1, Hongo, Bunkyo-ku, Tokyo 113-8421, Japan.

E-mail address: [yuuehara@juntendo.ac.jp](mailto:yuuehara@juntendo.ac.jp) (Y. Uehara).

All the initial MRSA isolates from the inpatients at Tokyo Metropolitan Children's Medical Center in Fuchu, Tokyo, Japan from March 2014 to April 2015 were used in this study. The isolates from outpatients and those collected within 48 h after admission were excluded as CA-MRSA. POT was performed by a multiplex PCR method (Cica Geneus Staph POT KIT; Kanto Chemical, Tokyo, Japan), according to the manufacturer's instructions. An isolate with a POT number of 106-9-93 was estimated as a USA300 strain, based on previous reports [10], and additional analyses were performed to confirm that these strains were USA300. Outbreak of USA300 was defined as at least one new isolation of USA300 from inpatients, because we had noticed USA300 as a cause of CA-MRSA infection in outpatients of the hospital and had been keeping vigilance against USA300 in inpatients [11]. Antimicrobial susceptibility was tested by the broth microdilution method, according to the Clinical Laboratory Standards Institute M100- S25 [12]. Investigation for the toxin genes and SCCmec typing were performed as previously described [11]. For the strains positive for the Panton–Valentine leukocidin gene (*lukS-lukF*), the presence of arginine catabolic mobile element (ACME)-*arcA* gene was investigated by PCR methods [11]. Pulsed-field gel electrophoresis (PFGE) by *SmaI* digestion was performed using USA300-0114, which was provided by the Centers for Disease Control and Prevention in the United States as a reference strain [13]. For the index strain, whole genome sequencing (WGS) was performed by Miseq (Illumina Inc. California, USA); the sequence type (ST) and *spa* type were determined by submitting the sequences to the MLST-1.8 server and *spa*Typer 1.0 websites, respectively, both of which were organized by the Center for Genomic Epidemiology in Denmark [14,15]. Ethics approval was obtained from the institutional review board of Tokyo Metropolitan Children's Medical Center, with approval number H27b-105.

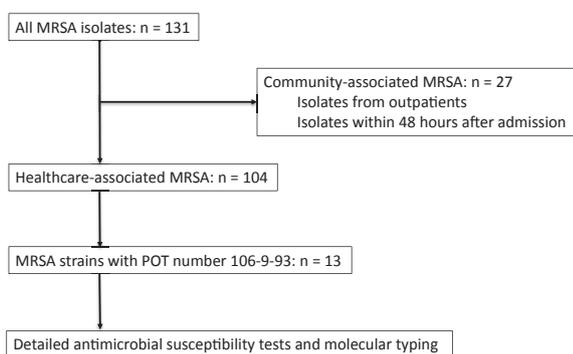
A total of 131 MRSA strains were identified during the study period, 27 of which were excluded as CA-MRSA (Fig. 1); finally, 104 MRSA strains were analyzed. Thirteen stains (12.5%) had a POT number of 106-9-93; these included 6 from nasal swabs, 6 from blood cultures and 1 from subcutaneous pus. All isolates were taken from patients in the NICU and were numbered as JICS141 to JICS153. All the strains were susceptible to vancomycin, clindamycin, sulfamethoxazole/trimethoprim and linezolid, but were resistant to levofloxacin and gentamicin. All the strains possessed the SCCmec IVa, PVL, and ACME-*arcA* genes, and the index strain JICS141 was typed as ST8 and t008. PFGE revealed that all the strains had an indistinguishable banding pattern with USA300-0114 (Fig. 2). These results confirmed that the MRSA strains with a POT number 106-9-93 were USA300 and caused the outbreak in the NICU. Number of



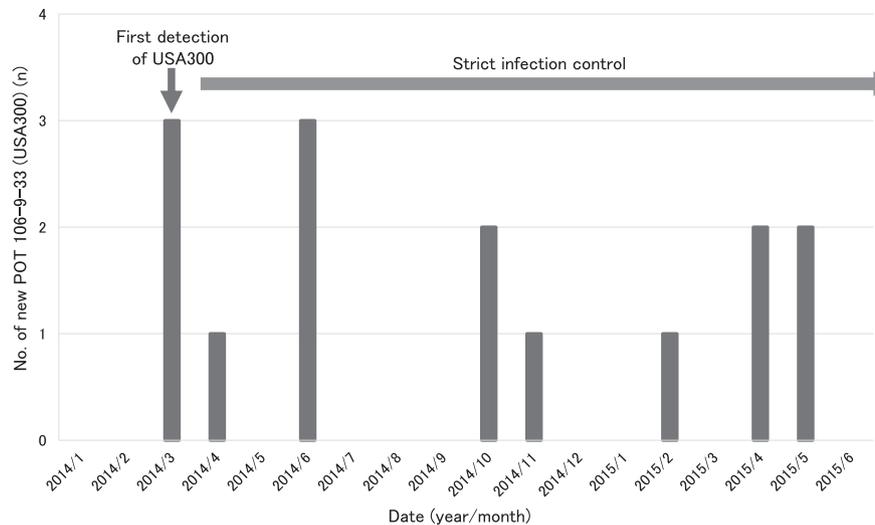
**Fig. 2.** Pulsed-field gel electrophoresis of MRSA isolates with POT number 106-9-93. All the MRSA isolates with POT number 106-9-93 from March 2014 to April 2015 showed undistinguishable pattern with USA300-0114. USA300-1440 was provided by the Center for Disease Control and Prevention in the United States. MWM, molecular weight marker; POT, polymerase chain reaction-based phage open reading frame typing.

the USA300 isolates from patients in NICU by month was shown in Fig. 3. Before the study period, neither MRSA strains with a POT number 106-9-93 had been isolated in the NICU, nor in the hospital. In March 2014, possible USA300 outbreak in the NICU was recognized with sudden appearance of 3 isolates with a POT number 106-9-93. Then, enhanced infection prevention and control was implemented; campaigns on hand hygiene, biweekly active surveillance for MRSA-negative infants with nasal swab, and pre-emptive contact precaution until the newly admitted neonates showed negative results for MRSA. In the study period, 41.9% (13/31) of MRSA isolated in NICU was USA300. The number of MRSA isolates with POT number 106-9-93 per a month waived from 0 to 3 as shown in Fig. 3, even though no MRSA with POT number 106-9-93 was isolated in June 2015. Strict infection prevention and control for all MRSA seemed necessary and was continued after the study period.

To our best knowledge, this was the first report of the outbreak of USA300 in a Japanese NICU and the successful detection of the strain by POT. We have previously reported on USA300 transmission among members of a Japanese family, but they were outpatients [11]. USA300 was previously reported to be disseminated in several Japanese hospitals and was suggested to potentially cause an epidemic [7]. The results of this study supported that USA300 might be one of the strains that can cause healthcare-associated infection in a Japanese NICU. In this study, all the USA300 isolates were found only in the NICU. Moreover, the index case with JICS141 was transferred from another hospital, although the screening culture upon admission to our hospital was negative for MRSA. No MRSA isolate before this case showed a POT number of 106-9-93. Therefore, it was difficult to identify the initial source of USA300 at our hospital. In 2008, nosocomial outbreak of USA300 in Japan was recognized in Okinawa [8]. Index patient was an adult with bullous pemphigoid who developed SSTI with USA300 after prednisolone treatment. Then, USA300 spread to 2 healthcare workers. In this report, the patient was thought to be a carrier of USA300 on admission, and isolation of the patient, strict contact precaution and hygienic care of equipment and environment successfully prevented patient-patient transmission. In our study, all



**Fig. 1.** Criteria for the selection of MRSA strains for detailed antimicrobial susceptibility tests and molecular typing. A total of 131 MRSA strains were identified during the study period, 27 of which were excluded as CA-MRSA; finally, 104 MRSA strains were analyzed. Thirteen stains had a POT number of 106-9-93. MRSA, methicillin-resistant *Staphylococcus aureus*; CA-MRSA, community-associated MRSA; POT, polymerase chain reaction-based phage open reading frame typing.



**Fig. 3.** Frequency of the patients having MRSA with a POT number 106-9-93 in the NICU by month. Before the study period, neither MRSA strains with a POT number 106-9-93, suggesting USA300, had been isolated in the NICU, nor in the hospital. The MRSA strain with a POT number 106-9-93, was firstly isolated from 3 patients in NICU in March 2014. The number of MRSA isolates with POT number 106-9-93 per a month waived from 0 to 3. MRSA, methicillin-resistant *Staphylococcus aureus*; NICU, neonatal intensive care unit; POT, polymerase chain reaction-based phage open reading frame typing.

MRSA with a POT number of 106-9-93 were isolated from the patients after 48 h of admission to the NICU. Transmission of USA300 from persons or environment to others in NICU would be caused by healthcare workers and guardians of neonates, but the source of USA300 could not be identified. In outbreak settings of new MRSA lineages in NICU, screening of healthcare workers, guardians and environment using POT might be useful to investigate the source of MRSA, though it had not been performed yet in this study.

POT was invented in Japan and can detect multiple open reading frames or sequences to characterize each strain using the multiplex PCR method; in fact, POT was reported to identify the major MRSA clones found in Japan [10]. Compared with phylogenetic analysis by PFGE or WGS, POT is advantageous in terms of simpler procedures, shorter turn-around time, and low cost for analysis, as well as the easier and faster detection of USA300 transmission in the wards. Actually, the cost to analyze one sample was approximately: 850 JPY for POT, 5,000 JPY for PFGE, and 10,000 JPY for WGS in our laboratory. However, notably, the use of whole genome information, such as that in PFGE or WGS, remains the gold standard analysis.

In this study, the impact of infection with USA300 on the outcome of NICU patients remains unclear because this study included only 7 patients (53.8%) of active USA300 MRSA infection and no one died at the end of study period. Further investigation about the clinical outcome of patients infected with USA300 in NICU is necessary.

In conclusion, POT was useful in recognizing this first MRSA outbreak of USA300 in a Japanese NICU and was advantageous in terms of swiftness, less cost and monitoring change of the epidemic MRSA lineage.

#### Authorship statement

All authors meet the ICMJE authorship criteria.

#### Conflicts of interest

Dr. Uehara reports grants from St. Luke's Life Science Institute, during the conduct of the study; personal fees from Taisho Toyama Pharm Co, personal fees from Astellas Pharm Inc, personal fees

from MSD, personal fees from Daiichi Sankyo Co, personal fees from Torii Pharm Co, personal fees from Meiji Seika Pharm Co, personal fees from Sumitomo Dainippon Pharma Co., Ltd., personal fees from Shionogi Pharm Co, personal fees from Novo Nordisk Pharm Co, outside the submitted work.; Dr. Okazaki reports grants from Tokyo metropolitan project research grant, outside the submitted work.; Dr. Horikoshi reports grants from Ministry of Health and Labor Welfare research grant, grants from Tokyo metropolitan project research grant, other from Janssen Pharm Co, other from Asahi Kasei Pharm Co, other from Fuji Film Pharm, personal fees from Abbvie GK, personal fees from Maruho Co, personal fees from JCR Pharm, personal fees from Taisho Toyama Pharm Co, personal fees from Astellas Pharm Inc, personal fees from Japan Vaccine Co, personal fees from Astellas Pharm Inc, personal fees from MSD, personal fees from BD Japan, personal fees from Pfizer Japan Inc, outside the submitted work.; Dr. Hiramatsu reports grants from Ministry of Education, Culture, Sport, Science, and Technology, Japan, during the conduct of the study; grants from Japan Agency for Medical Research and Development, personal fees from Zen-yaku Kogyo Co., Ltd., outside the submitted work.

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