Neonatal *Staphylococcus aureus* acquisition at a tertiary intensive care unit

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ABSTRACT

Background

In this study, we explored the role of colonization in health care workers (HCWs) in transmission of methicillin-susceptible *Staphylococcus aureus* (MSSA) to neonates at a level IV neonatal intensive care unit (NICU).

Methods

All available screening and clinical MSSA isolates from the period March 2015 through April 2016, isolated from HCWs and neonates at the level IV NICU, were included. MSSA isolates were initially genotyped using *spa* typing and for the most prevalent *spa* types, whole-genome sequencing (WGS) was performed.

Results

From March 2015 until April 2016, 159 neonates and 115 HCWs were found positive for MSSA, and all isolates were typed by means of *spa* typing. Twenty-three *spa* types were found in both HCWs and neonates. Within the most prevalent *spa* types (t002, t015 and t2787), 4 WGS clusters of genetically indistinguishable MSSA isolates were found in which 4 HCWs and 35 neonates were involved. A total of 10 neonates included in the 4 WGS clusters suffered from bacteremia.

Conclusion

We showed that HCWs carried the same MSSA isolates as those found in neonates, and that HCWs might serve as a reservoir for transmission of MSSA to neonates. Ten neonates suffered from a bacteremia caused by a MSSA previously detected in a HCW.

INTRODUCTION

Neonates admitted to an intensive care unit (NICU) are with their low gestational age, low birth weight, a microbiome under construction, and with immaturity of organ systems prone to develop health care-associated infections (HAIs), in particular bloodstream infections [1]. In neonates, *Staphylococcus aureus* (*S. aureus*) is one of the three most commonly found bacteria in the blood [2-4]. In adults, it is known that the majority of *S. aureus* bacteremias are caused by the endogenous strain [5], however, in neonates, with their immature microbiome, the cause and effect are unknown. There are many studies published that focus on transmission of methicillin-resistant *S. aureus* (MRSA) on NICUs that resulted in colonization or infection with MRSA [6-12]. However, there is less data available on transmission of methicillin-susceptible *S. aureus* (MSSA) on NICUs, whereas this could at least have a similar impact given the number of infections [13-17]. In this study, we explored the possibility of health care workers (HCWs) being a reservoir of transmission of MSSA to neonates at a level IV NICU in a 14-month period of time. Whole-genome sequencing (WGS) was used as a typing method to detect genetic relatedness of the isolates.

METHODS

Population

The NICU of Erasmus MC-Sophia, Rotterdam, The Netherlands, is a level IV 27-beds facility. It is divided into 4 units with 6-8 beds each. About 750 neonates are admitted annually, and nearly 40% of those are below 32 weeks of gestational age, with the majority being born in the hospital.

Ethics statement

Because this was a retrospective observational study using anonymized patient data collected during routine clinical practice, informed consent was not mandatory, according to the Dutch Medical Research Involving Human Subjects Act (WMO). The Institutional Ethics Review Board of the Erasmus MC reviewed the study protocol and provided an exemption from formal ethical assessment (MEC-2015-306) based on the non-interventional design. The study was carried out in accordance with the current ethical guidelines for epidemiological research.

Screening

At the level IV NICU in the Erasmus MC-Sophia, Rotterdam, The Netherlands, all neonates are screened weekly by swabbing the throat and the rectum. Additional screening cultures for *S. aureus*, from neonates as well as HCWs, were taken in the period from March 2015

through April 2016, because of 3 separate MSSA outbreaks at the NICU, not described in the present study. HCWs who took care of neonates involved in the outbreaks and neonates who shared the same unit, in the same time period, with neonates involved in the outbreaks, were considered contacts. For HCWs who were contacts of neonates involved in the outbreaks, the nasal and the throat swabs were collected. For neonates who were contacts, additional rectal, nasal or perineal swabs were collected. In case of suspicion of infection, clinical samples were collected as part of routine diagnostics. Clinical samples included blood cultures, sputum, skin cultures, wound fluid or cultures from specific body sites (eg, eye or ear). All *S. aureus* isolates derived from neonates and HCWs, obtained from routine diagnostics as well as from contact investigations, are used in this study. Contact investigation is defined as an active screening to detect transmission between HCWs and neonates.

Culture, identification, and susceptibility of *S. aureus* isolates

Swabs and clinical samples were cultured on Trypticase Soy Agar II with 5% Sheep Blood (BD, Heidelberg, Germany). After 24-48 hours of incubation at 35°C, plates were screened for *S. aureus* based on colony morphology and MALDI-TOF analysis (Maldi-tof MS system, Bruker). *S. aureus* isolates were stored at – 20 °C or – 80 °C until use. The VITEK 2 system (bioMérieux, Marcy l'Etoile, France) was used for antibiotic-susceptibility testing.

***Spa* typing of MSSA isolates**

DNA was isolated from pure colonies using the automated MagNA Pure 96 platform in combination with the MagNA Pure DNA and Viral Nucleic Acid Small Volume Kit (Roche diagnostics, Almere, the Netherlands). PCR reactions were performed in 25-μl reactions using 1 μL of isolated DNA in 1x FastStart PCR Master (Roche) and 0.5 μM of both forward primer (5'-AACAACGTAACGGCTTCATCC-3') and reverse primer (5'- GCTTTTGCAATGTCATTTACTG-3'). Thermal cycling consisted of an initial denaturation step for 10 min at 95°C, followed by 35 cycles of 30s at 95°C, 30s at 60°C and 1 min at 72°C. After a final extension step of 10 min at 72°C, reactions were cooled to room temperature. Five μl of PCR product was analyzed on agarose gel to confirm amplification. The remainder of the PCR product was treated with 2 μL of ExoSAP-IT (Isogen Life Science, De Meern, the Netherlands) for 15 min at 37°C, following inactivation for 15 min at 80°C. Amplicon sequencing was performed by BaseClear (Leiden, the Netherlands) using the forward amplification primer as sequencing primer. Electropherograms were analyzed and interpreted using the *spa* typing plugin in BioNumerics v7.6 software (Applied Maths, Sint-Martens-Latem, Belgium).

WGS

The selection of MSSA isolates for WGS was based on the results of *spa* typing. MSSA isolates were subjected to next-generation sequencing (NGS) by generating PE125 reads on an Illumina HiSeq 2500 (BaseClear, Leiden, the Netherlands). Sequence data was assembled

using CLC Genomics Workbench v11 software (Qiagen, Hilden, Germany) and analyzed using the available *S. aureus* schemes in SeqSphere v 4.1.9 software (Ridom, Münster, Germany).

Definitions

Possible transmission: if the MSSA isolate from the HCW-to-neonate or neonate-to-neonate are genetically indistinguishable determined by WGS.

WGS cluster: a cluster of indistinguishable MSSA isolates defined as a difference of less than 12 alleles on the core genome [18].

RESULTS

In the period from March 2015 until April 2016, 159 neonates and 115 HCWs were found positive for MSSA and all isolates were typed by means of *spa* typing. In the 159 neonates, a total number of 60 different *spa* types were found. In total 255 positive MSSA cultures were taken from the 159 neonates of which 59% were screening cultures (31% rectum, 17% nose, 8% throat and 3% perineal cultures). The other positive MSSA cultures originated from blood (11%), skin (7%), sputum (7%), wound fluid (6%), eye (4%), ear (1%) or origin unknown (4%). In 115 HCWs, 71 different *spa* types were detected. Cultures from HCWs originated from nose or throat. Fourteen neonates and 7 HCWs were colonized, with 2 morphologically different MSSA with different *spa* types. Twenty-three *spa* types were found in both HCWs and neonates. In these 23 shared *spa* types, 117 of 159 (74%) neonates and 67 of 115 (58%) HCWs were included. Table 1 shows the number of cultures, the number of positive blood cultures in neonates, and the number of HCWs and neonates in the 23 shared *spa* types.

Genetic relatedness based on WGS

As *spa* typing has less discriminatory power than WGS, WGS was performed on MSSA isolates of the 3 most prevalent *spa* types. *Spa* types t002, t015 and t2787 were selected to perform WGS. These 3 *spa* types involved 29.3% (n = 54; 40 neonates and 14 HCWs) of all neonates and HCWs (Table 1), and represented 11 out of the total number of 20 (55%) positive blood cultures in neonates (Table 1). One MSSA isolate per individual was used for WGS. Figure 1 shows that 4 different WGS clusters were identified that considered transmission possible. A total of 4 HCWs and 35 neonates, of whom 10 neonates suffered from a neonatal bacteremia, were involved in 4 different WGS clusters. Consequently, the MSSA isolates from 10 of 14 HCWs and 5 of 40 neonates could not be clustered by WGS. Figure 2 shows the epicurves of WGS clusters A-D. WGS clusters A, B and D included neonates as well as HCWs. WGS cluster A (*spa* type t2787) was the largest cluster and included only 1 HCW who was cultured positive in April 2015. A total of 19 out of 20 neonates included in this WGS cluster with indistinguishable MSSA were cultured positive in the period April 2015 through

Table 1. The 23 shared *spa* types with size of each *spa* type cluster and the number of cultures and positive blood cultures in neonates.

spa type	No. of neonates	No. of cultures	No. of pos. blood cultures	No. of HCWs	No. of cultures
t002 ◊	9	13	4	8	9
t005	1	1	0	1	1
t008	4	4	0	3	4
t012	3	3	0	4	5
t015 ◊	10	14	3	5	8
t026	2	2	0	1	1
t065	1	1	0	4	5
t084	5	19	1	7	7
t091	8	12	0	3	3
t127	8	15	1	2	3
t189	6	9	1	5	12
t223	12	17	2	7	10
t230	3	3	1	3	6
t250	5	5	0	2	2
t491	3	6	0	1	1
t571	5	6	1	3	6
t659	5	13	0	3	5
t864	3	4	0	1	4
t16721	1	1	1	1	2
t16723	2	3	0	1	2
t16731	1	2	0	1	1
t16779	6	6	1	1	3
t2787 ◊	21	25	4	1	2
Total	124*	184	20	68**	102

HCWs, health care workers; pos., positive

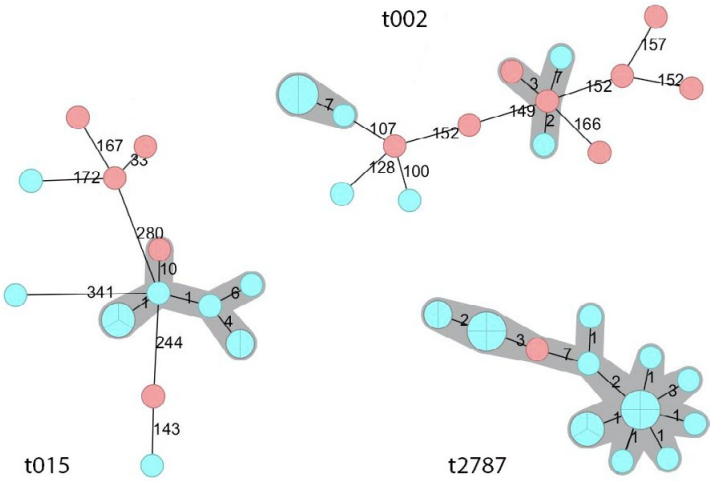
* A total of 7 neonates were positive for 2 MSSA isolates with different *spa* types.

** One HCW was positive for 2 MSSA isolates with different *spa* types.

◊ *spa* type cluster used for whole-genome sequencing.

November 2015. Four neonates suffered from a bacteremia with a MSSA isolate from WGS cluster A. In WGS cluster B (*spa* type t002), 2 HCWs were cultured positive in April 2015, and 2 neonates were cultured positive. In 1 of these neonates, the MSSA isolate responsible for WGS cluster B caused a bacteremia. WGS cluster C (*spa* type t002) included neonates only. Three of the total 5 neonates had a positive blood culture for the MSSA isolate responsible for WGS cluster C. WGS cluster D (*spa* type t015) included 1 HCW cultured positive in April 2015, and 8 neonates of whom 2 suffered from a bacteremia.

Figure 1. Whole-genome sequencing of 3 clusters of *spa* types revealed 4 new WGS clusters, with each WGS cluster representing a genetically different MSSA isolate.



Minimum spanning tree representing cgMLST analysis of 54 selected MSSA isolates based on *spa* typing (as indicated). Each circle represents a different genotype. Numbers correspond to the number of allelic differences between the genotypes. Segments within circles represent the number of isolates of that genotype. Clusters of genotypes with <12 differences are indicated with a grey background. Colors indicate the origin of the isolates: patient (blue) and health care worker (red). WGS, whole-genome sequencing.

DISCUSSION

By using WGS we showed that HCWs might serve as a reservoir for transmission of MSSA to neonates on a level IV NICU. Of the 4 HCWs and 35 neonates, 10 neonates suffered from a neonatal bacteremia and were involved in 4 different WGS clusters. By analyzing WGS data, we conclude that 10 of 20 episodes of MSSA bacteremia, of which the MSSA isolates belonged to a *spa* type found in both neonates and HCWs, were due to the presence of isolates that were already present in HCWs or neonates admitted earlier and possibly also from the environment. Data concerning MSSA transmission from HCW to neonate are scarce since normally no contact investigation is performed when a neonate is positive for MSSA, in contrast with MRSA findings. In three studies MSSA transmission from HCW to patient was described. Gomez-Gonzalez et al. showed, albeit by use of Pulse Field Gel Electrophoresis (PFGE), a technique with less discriminatory power, that HCWs acted as a reservoir for neonates that later on developed a MSSA sepsis [17]. In another study, a HCW proved to be the source of an outbreak in neonates with MSSA bullous impetigo by Raman spectroscopy typing [19]. In addition, Price et al. showed that patients on an adult intensive care unit got colonized and infected with genetically identical *S. aureus* strains transmitted via patients, the environment and health care workers. Whole-genome sequencing was used to prove transmission [20].

Figure 2. Epicurves of WGS clusters A – D.

Cluster A

HCW	---------	□	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------
neonate	○	○○○○○○○○	○○○○	---------	◊ ○○	---------	---------	◊ ○	◊◊	---------	---------	---------	---------	---------
month	M	A	M	J	J	A	S	O	N	D	J	F	M	A

◊ neonate - positive blood culture for MSSA
○ neonate - other culture positive for MSSA
□ HCW – nose or throat positive for MSSA

time (March 2015 – April 2016)

Cluster B

HCW	---------	□□	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------
neonate	---------	---------	---------	---------	◊	---------	---------	---------	---------	---------	---------	---------	---------	○
month	M	A	M	J	J	A	S	O	N	D	J	F	M	A

◊ neonate - positive blood culture for MSSA
○ neonate - other culture positive for MSSA
□ HCW – nose or throat positive for MSSA

time (March 2015 – April 2016)

Cluster C

HCW	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------
neonate	---------	○	---------	---------	---------	---------	---------	---------	---------	---------	---------	◊	◊ ○	◊
month	M	A	M	J	J	A	S	O	N	D	J	F	M	A

◊ neonate - positive blood culture for MSSA
○ neonate - other culture positive for MSSA

time (March 2015 – April 2016)

Cluster D

HCW	---------	□	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------
neonate	---------	---------	○○	◊ ○	◊	---------	---------	---------	---------	○	○	○	---------	---------
month	M	A	M	J	J	A	S	O	N	D	J	F	M	A

◊ neonate - positive blood culture for MSSA
○ neonate - other culture positive for MSSA
□ HCW – nose or throat positive for MSSA

time (March 2015 – April 2016)

As there is obviously no patient-to-patient contact at the NICU, we hypothesize that transmission was by direct contact of contaminated hands of the HCWs (eg, by touching the nose or nose picking) or, that the MSSA isolates of the HCWs survived in the environment and then were transmitted by hand after touching the environment. This could also explain why WGS cluster C prolonged for 12 months whereas no HCW was cultured positive in that period for this specific MSSA isolate. Other reported risk factors for transmission are, besides environment, overcrowding of patients and understaffing [10,20,21].

Our study has several limitations. This study is retrospective and performed in a single center. HCWs were not routinely cultured and seldom more than once. As the culture moments were not defined in advance no conclusions can be drawn on whether or not the HCWs were actually *S. aureus* persistent or intermittent carriers. No other carriage sites, other than nose and throat, from HCWs were cultured therefore the number of HCWs carrying *S. aureus* could have been an underestimation. Neither the environment nor the parents were cultured for MSSA; this could have given more clues about other reservoirs and route(s) of transmission. In addition, if MSSA was also detected in the environment, we could have used disinfection measures to stop ongoing transmission. If parents proved to be a reservoir of MSSA, probably transmission from the neonate to the HCW may have occurred, instead of vice versa, as we assume now. However, this would not explain persistence and transmission of the same MSSA isolate over a period of time and to more than one neonate. Demographic data are not included because, regardless of the medical history of the patient, transmission of MSSA will probably still occur. Lastly, *spa* typing proved to have less discriminatory power to show genetic relatedness compared to WGS. The initial 3 most prevalent clusters of *spa* types revealed 4 new clusters based on WGS, of which 2 WGS clusters were even included in just 1 of the *spa* type clusters (t002). Therefore, regarding future transmission or epidemiology studies, we would not advise the use *spa* typing as a typing method. Finally, our data could be an underestimation of the problem as we performed WGS for only 3 clusters of *spa* types.

CONCLUSIONS

In conclusion, we showed that HCWs might be an important reservoir and link in the transmission of MSSA to neonates. Ten neonates developed a MSSA bacteremia with a nosocomial acquired MSSA isolate that was previously detected in a HCW. Although the route of transmission remains to be elucidated, these data clarify that additional infection prevention measures are urgently needed. Although hand hygiene is well performed at the NICU when observed (directly unobtrusively) [22], one might still consider additional training programs on hand hygiene. Other options are more intensive daily cleaning of the environ-

ment or wearing a protective mask by the HCW in order to prevent touching the nose, the niche of *S. aureus*, and to prevent spreading of droplets.

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