Whole-genome sequencing to explore nosocomial transmission and virulence in neonatal methicillin-susceptible
Staphylococcus aureus bacteremia

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Abstract

Background: Neonatal Staphylococcus aureus (S. aureus) bacteremia is an important cause of morbidity and mortality. In this study, we examined whether methicillin-susceptible S. aureus (MSSA) transmission and genetic makeup contribute to the occurrence of neonatal S. aureus bacteremia.

Methods: A retrospective, single-centre study was performed. All patients were included who suffered from S. aureus bacteremia in the neonatal intensive care unit (NICU), Erasmus MC-Sophia, Rotterdam, the Netherlands, between January 2011 and November 2017. Whole-genome sequencing (WGS) was used to characterize the S. aureus isolates, as was also done in comparison to reference genomes. Transmission was considered likely in case of genetically indistinguishable S. aureus isolates.

Results: Excluding coagulase-negative staphylococci (CoNS), S. aureus was the most common cause of neonatal bacteremia. Twelve percent (n = 112) of all 926 positive blood cultures from neonates grew S. aureus. Based on core genome multilocus sequence typing (cgMLST), 12 clusters of genetically indistinguishable MSSA isolates were found, containing 33 isolates in total (2–4 isolates per cluster). In seven of these clusters, at least two of the identified MSSA isolates were collected within a time period of one month. Six virulence genes were present in 98–100% of all MSSA isolates. In comparison to S. aureus reference genomes, toxin genes encoding staphylococcal enterotoxin A (sea) and toxic shock syndrome toxin 1 (tsst-1) were present more often in the genomes of bacteremia isolates.

Conclusion: Transmission of MSSA is a contributing factor to the occurrence of S. aureus bacteremia in neonates. Sea and tsst-1 might play a role in neonatal S. aureus bacteremia.

Keywords: Staphylococcus aureus, Bacteremia, Whole-genome sequencing, Neonatal intensive care unit, Transmission
**Introduction**

*Staphylococcus aureus* (*S. aureus*) is a well-established nosocomial pathogen that causes multiple types of neonatal infections [1, 2]. Invasive *S. aureus* infections in neonates (e.g., bacteremia) are common in very low birth weight (VLBW) infants, which makes this bacterial species one of the most important pathogens in neonatal intensive care units (NICU) [3–5]. A significant risk factor for *S. aureus* bacteremia in VLBW infants is the presence of intravascular catheters, which are frequently required [6–8]. In addition, *S. aureus* bacteremia can result in severe complications such as endocarditis and osteomyelitis [5, 9, 10]. All-cause mortality among neonates suffering from *S. aureus* bacteremia varies between 10 and 20% [7, 11]. So there is an urgent need to prevent this infection. To prevent *S. aureus* bacteremia in neonates, it is important to know the factors contributing to the high frequency and severity of this infection.

Previously, the virulence factors *tsst-1* and *sea* were implicated to play a role in *S. aureus* bacteremia [12–14]. Furthermore, transmission of *S. aureus* might contribute to the high frequency of bacteremia. Outbreaks of methicillin-resistant *S. aureus* (MRSA) at the NICU are described and relatively easy to detect [15–18]. Meanwhile, the detection of methicillin-sensitive *S. aureus* (MSSA) outbreaks seems to be more difficult, excluding outbreaks in patients who suffer from a skin infection [19–22]. In this study, whole-genome sequencing (WGS), the typing method with the highest discriminatory power, was used to determine whether MSSA transmission and genetic makeup, contribute to the occurrence of neonatal *S. aureus* bacteremia.

**Methods**

**Population**

The NICU of Erasmus MC-Sophia, Rotterdam, the Netherlands, is a level IV, 27-beds facility. It is divided into four units with six to eight beds each. Per year, about 750 neonates are admitted. Nearly 40% of them are below 32 weeks of gestation and were in majority born in this hospital.

**Screening**

We included neonates with a presumed infection, of whom blood cultures were obtained between January 2011 and November 2017 that showed to be positive for *S. aureus*. Clinical data concerning gender, gestational age, birth weight and survival were obtained from patient records.

**S. aureus isolates**

Blood from neonates was cultured in BACTEC plus PEDS aerobic bottles and incubated in the Bactec FX (BD, Heidelberg, Germany). In case of positive blood cultures, plates were inoculated and, after 16–24 h of incubation at 37 °C, screened for *S. aureus* based on colony morphology. Identification was performed by means of a latex agglutination test (Slidex Staph Plus, bioMérieux, Marcy-l’Etoile, France) and/or via matrix-assisted laser desorption/ionisation, time-of-flight, mass spectrometry (MALDI-TOF MS system, Bruker). *S. aureus* isolates were stored at −20 °C or −80 °C until use. The VITEK 2 system (bioMérieux) was used for antimicrobial susceptibility testing (AST).

**Whole-genome sequencing**

**Transmission**

*S. aureus* isolates were processed according to the bioMérieux EpiSeq™ V1 programme and sent to LGC Genomics GmbH (Berlin, Germany) for next-generation sequencing (NGS). We used Illumina chemistry, which generated paired end 2 × 150 bp reads. Sequences were assembled using the proprietary built-in assembler from CLC Genomics Workbench v11 software (Qiagen, Hilden, Germany) with default parameters. We analysed them by means of the available *S. aureus* core genome multilocus sequence typing scheme (cgMLST) [23] in BioNumerics 7.6.3 (bioMérieux, Sint-Martens-Latem, Belgium) which contains 1861 loci. Allele calling was performed using two algorithms, one based on the assembly using a BLAST approach (assembly-based calling) and one based on the trimmed sequencing data using a kmer based approach (assembly-free calling). A consensus of both algorithms was used to assign final allele calls: when both algorithms were in agreement or when an allele call was made by only one of the algorithms, the allele call was considered in the consensus. However, when both algorithms were in disagreement, the allele call was not considered in the consensus. Both allele calling algorithms were executed using default parameters. Conventional MLST types were inferred in silico from the WGS data. To this end, the seven MLST loci were identified using the sequence extraction tool and the MLST plugin from BioNumerics 7.6.3 that is synchronized to the pubMLST.org public repository (accession date: April 5, 2019). For the visualisation of the genetic relatedness between the isolates, we used a minimum spanning tree for the cgMLST data. The MST was generated using default parameters, and no re-sampling was performed. Isolates containing less than 12 allelic differences in the *S. aureus* core genome were considered genetically indistinguishable [23]. We defined a cluster as more than two genetically indistinguishable isolates and, within a cluster, considered transmission of *S. aureus* likely. To further validate the results based on the cgMLST approach, as additional method, we evaluated transmission events using a SNP based approach (Additional file 1: Table S1).
Virulence
The presence of virulence genes was assessed, using the
sequence extraction tool in BioNumerics 7.6.3. Extrac-
tion parameters (percentage coverage and identity) were
individualised to accommodate for the different levels of
sequence diversity within and between the virulence
genes. Anticipating problems upon assembling virulence
genes containing repetitive motifs (sdrA, –B and -C, clfA
and –B, cna, sasG) using the short read sequence data,
only the largest non-repetitive part of these genes was
used for quering. In order to obtain data from a general
S. aureus population, the prevalence of virulence genes
was also assessed by means of the available genomic se-
quences in the Refseq Genome Database, using the
BLAST interface (https://blast.ncbi.nlm.nih.gov/Blast.
cgi). This database contained 10,288 S. aureus genomes
at the time of analysis. Virulence gene-specific search parameters were used as discussed above. Role and func-
tion of the S. aureus virulence genes were described in
more detail earlier [12, 24]. An overview of analysed
virulence genes, their role, search parameters and query
sequence are shown in Additional file 2: Table S2.

Results
Patient characteristics
After coagulase-negative staphylococci (CoNS), MSSA
was the most frequent causative pathogen of bacteremia
in neonates. Several species of CoNS were isolated from
neonatal blood, but they were considered to be one
group. Twelve percent (n = 112) of 926 positive blood
cultures from neonates (one blood culture per episode
per patient), taken in the period January 2011 – Novem-
ber 2017, were positive for MSSA. Fifty-nine of the 112
neonates (52.7%) with MSSA bacteremia were male. The
median (interquartile range) for gestational age and birth
weight were 26 3/7 (25 1/7–30) weeks and 880 (680–
1150) grams, respectively. The onset of all episodes of
MSSA bacteremia occurred 72 h after birth, at a median
postnatal age of 10 (7–19) days. The overall mortality
among the included 112 patients was 20.5% while 11 of
these 23 neonates died of MSSA septicemia.

Genetic relatedness
One hundred and four MSSA isolates from the total of 112
neonatal bloodstream isolates (93%) were available and
therefore included for WGS (including only the first isolate
per patient). Based on WGS, a total of 23 classical MLST
types were identified. The most predominant MLST types
were ST5 and ST45 (for both n = 16). For 11 MSSA isolates
a novel MLST type was found. To assess the genetic re-
latedness between the 104 isolates based on the more dis-
criminatory cgMLST scheme, we visualised the number of
allelic differences of the isolates in Fig. 1. Twelve cgMLST
clusters of genetically indistinguishable isolates were
observed, containing a total of 33 isolates (2–4 isolates per
cluster). In seven of these cgMLST clusters, at least two of
the identified MSSA isolates were collected within a time
period of one month. In two cgMLST clusters, all MSSA
isolates were found within a time period of one year, but
the shortest time interval between isolates of two neonates
was forty days. In the other three cgMLST clusters, there
was a time interval of more than one year between cultur-
ing the MSSA bloodstream isolates of two neonates. The
SNP approach confirmed our results based on the cgMLST
approach (Additional file 1: Table S1).

S. aureus virulence genes
An overview of virulence genes present in the 104 MSSA
isolates is provided in Table 1. Of the immunomodula-
tory proteins, staphylococcal complement inhibitor (scin)
was present in 100% of all bloodstream isolates. Alpha-
haemolysin (hla) was present in 99% of the isolates. We
also found a 98–100% presence of the MSCRAMMs
clumping factors A and B (clfA, clfB), immunodominant
surface antigen A (isaA) and iron-responsive surface de-
terminants A and H (isdA, isdH). When compared to a
reference population of S. aureus genomes, a few obser-
vations stand out. Remarkably, staphylococcal enteroto-
oxin A (sea) and toxic shock syndrome toxin 1 (tsst-1)
were, respectively, 2.6 and 3.2 times more prevalent
among the 104 neonatal bloodstream isolates, relative to
the reference genomes. Likewise, staphylococcal enteroto-
oxin h (seh) was 3.4 times more prevalent although, in
absolute numbers, this involved only a few isolates (6/
104 versus 173/10288). For the other virulence genes, no
such increases were detected (Table 1).

Discussion
At our level IV neonatal intensive care unit, as in many
centres [3–5], S. aureus is a frequent cause of neonatal
bacteremia. In our study, we explored the role of MSSA
transmission and the possible contribution of virulence
genes. By using WGS, 12 different cgMLST clusters of
MSSA isolates were found. Seven of these twelve
cgMLST clusters included at least two MSSA isolates,
cultured from blood of neonates within one month, indi-
cative for transmission. Transmission should therefore
be considered as a contributing factor for the frequent
occurrence of neonatal S. aureus bacteremia, as was re-
cently described by Rouard et al. [13]. Although it seems
reasonable to assume that transmission, irrespective of
the source, can only occur through the hands of health-
care workers (HCWs), we did not prove this, since we
did not culture the environment, nor the HCWs or par-
ents. Still, general measures such as improvement of the
current (daily) cleaning, disinfection procedures as well
as hand hygiene, will be likely to help. It was already
proven that neonatal hospital-acquired infections could
in part be prevented by strict infection control measures [8, 25, 26]. In addition, reinforcement of the implementation of central-line bundles has the potential to reduce the incidence of central line-associated bloodstream infections (CLABSI); although these bundles are already implemented, compliance can still be improved and additional measures can be explored [27].

Besides transmission, it was determined whether the presence of certain virulence factors is associated with neonatal S. aureus bacteremia. Since it was difficult to define a suitable control population of neonates, we chose to compare neonatal S. aureus bacteremia isolates to all available S. aureus genomes from the Refseq Genome Database (N = 10,288 at the time of analysis). Remarkably, the genes sea and tsst-1 were found a factor 2.6 and 3.2 times more often in the MSSA bloodstream isolates, compared to the reference genomes in the Refseq Genome Database. The overrepresentation of tsst-1 could not be explained by the frequent presence of MLST ST5 and ST45 in our isolates collection, since tsst-1 was not...
associated with these sequence types. On the other hand, 11 of the 25 isolates carrying see were found in ST5 isolates. Still, this cannot be the full explanation for finding an association between sea and neonatal MSSA bacteremia. Many studies have been executed on S. aureus toxins and their pathogenic roles, particularly on sea and tsst-1. Previously, it was described that antibody responses to these two specific toxins were higher in patients with S. aureus bacteremia, compared to control patients [12]. In addition, in a recent publication about a NICU MSSA outbreak, tsst-1 and especially sea were found in bloodstream isolates, compared to colonisation isolates [13]. Another review article describes the association of these toxins with bacteremia [14]. Therefore, this may suggest that sea and tsst-1 might play a role in the pathogenesis of S. aureus bacteremia. The other virulence genes were present in virtually all study isolates, but in virtually all reference genomes as well (Table 1).

Our study has its limitations. It was performed retrospectively, in a single centre. We considered less than 12

### Table 1 Presence of virulence genes in neonatal S. aureus isolates compared to reference genomes

| genes | neonatal isolates (%) | refseq (%) | genes | neonatal isolates (%) | refseq (%) |
|-------|-----------------------|------------|-------|-----------------------|------------|
| sea   | 24.0                  | 9.4        | cna   | 51.0                  | 34.6       |
| seb   | 48.0                  | 5.9        | eap-map | 64.4                | 93.7       |
| sec   | 18.3                  | 10.8       | ebp   | 95.2                  | 95.2       |
| sed   | 10.6                  | 8.8        | fnbpA | 66.3                  | 75.2       |
| seg   | 51.9                  | 55.3       | fnbpB | 65.4                  | 75.0       |
| seh   | 5.8                   | 1.7        | sdrC  | 91.3                  | 96.3       |
| sei   | 55.8                  | 55.2       | sdrD  | 50.0                  | 74.9       |
| sei   | 10.6                  | 11.1       | sdrE  | 89.4                  | 85.0       |
| sek   | 3.8                   | 19.0       | etb   | 77.9                  | 94.7       |
| sef   | 18.3                  | 10.8       | icaA  | 100.0                 | 97.8       |
| sem   | 55.8                  | 55.6       | icaB  | 89.4                  | 98.3       |
| sen   | 45.2                  | 55.2       | icaC  | 99.0                  | 97.8       |
| seo   | 58.7                  | 49.1       | icaD  | 100.0                 | 98.0       |
| sep   | 4.8                   | 19.1       | icaR  | 95.2                  | 97.7       |
| seq   | 4.8                   | 19.1       | isaA  | 100.0                 | 98.3       |
| ser   | 10.6                  | 10.9       | isaA  | 100.0                 | 97.6       |
| ses   | 0.0                   | 0.3        | isdH  | 100.0                 | 97.5       |
| set   | 0.0                   | 0.3        | sasG  | 54.8                  | 56.7       |
| seu   | 51.9                  | 54.8       | edn   | 1.0                   | 1.4        |
| sey   | 1.0                   | 5.8        | etA   | 1.0                   | 1.0        |
| aur   | 100.0                 | 98.2       | etB   | 0.0                   | 0.2        |
| coa   | 70.2                  | 83.0       | etD   | 1.0                   | 0.8        |
| geh   | 87.5                  | 97.5       | hla   | 99.0                  | 97.1       |
| hysA  | 98.1                  | 96.5       | hlb   | 100.0                 | 95.1       |
| sak   | 73.1                  | 78.5       | hld   | 90.4                  | 98.0       |
| sspA  | 99.0                  | 98.1       | hlgA  | 96.2                  | 97.6       |
| sspB  | 91.3                  | 98.1       | hlgB  | 100.0                 | 97.8       |
| sspC  | 100.0                 | 98.3       | hlgC  | 98.1                  | 97.5       |
| vWbp  | 78.8                  | 98.1       | lukD  | 49.0                  | 66.5       |
| adsA  | 96.2                  | 98.1       | lukE  | 49.0                  | 65.9       |
| chp   | 68.3                  | 67.2       | lukF  | 0.0                   | 18.8       |
| sbi   | 100.0                 | 97.7       | lukM  | 0.0                   | 0.7        |
| scn   | 98.1                  | 98.2       | lukS  | 0.0                   | 19.0       |
| clfA  | 98.1                  | 97.4       | tsst-1 | 18.3                | 5.8        |
| clfB  | 100.0                 | 97.8       |
allelic differences in the \textit{S. aureus} core genome as indistinguishable, as described by Leopold et al. for MRSA outbreaks [23]. Still, it is a matter of debate which cut-off should be used to define MSSA isolates as indistinguishable. If the cut-off had been set at 20 alleles [28], this would have led to 10 larger instead of 12 indistinguishable MSSA clusters, which would not have changed the conclusion regarding transmission. Additional studies are needed to define a clear cut-off. Finally, we compared neonatal isolates to a large number of reference genomes, but these originate from several countries, several clinical sites and patients of all ages. It would have been ideal if the reference genomes had originated from colonized but not infected neonates, admitted to the same NICU, in the same time period.

Conclusions

In conclusion, transmission of MSSA seems a contributing factor to the occurrence of \textit{S. aureus} bacteremia in neonates. The possibility of MSSA transmission in neonatal intensive care should be explored to prevent this invasive and serious infection. The exact role of \textit{sea} and \textit{tsst-1} warrants further investigation.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13756-020-0699-8.

Additional file 1: Table S1 Whole-genome Single-Nucleotide Polymorphism analysis

Additional file 2: Table S2 \textit{S. aureus} virulence genes

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Author’s contributions

BS contributed to the study design, collected, analysed and interpreted the data, and drafted the initial manuscript. NV conceptualised and designed the study, collected, analysed, interpreted and supervised the data collection and critically reviewed and revised the manuscript for intellectual content. MV contributed to the study design, collected data, and critically revised and reviewed the manuscript for intellectual content. RK IR contributed to the study design, collected data, and critically reviewed and revised the manuscript for intellectual content. DDC CK contributed to the study design, collected, analysed and interpreted the data, and critically revised and reviewed the manuscript for intellectual content. AVB WG conceptualised and designed the study, interpreted the data and critically reviewed and revised the manuscript for intellectual content. The author(s) read and approved the final manuscript.

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Availability of data and materials

The data generated and analysed in this study are included in the current article.

Ethics approval and consent to participate

Because this was a retrospective observational study in which anonymised patient data were used, 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.

Consent for publication

Not applicable.

Competing interests

All other authors declare that there are no competing personal or institutional interests.

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References

1. Jeong JS, Jeong JS, Choi EO. Nosocomial infection in a newborn intensive care unit (NICU), South Korea. BMC Infect Dis. 2006;6:103.
2. Reichert F, Pieren B, Geffer C, Gastroi Neer P, Buhrer C, Schwab F. Pathogen-specific clustering of nosocomial blood stream infections in very preterm infants. Pediatrics. 2016;137.
3. Ericson JE, Popoola VO, Smith PB, Benjamin DK, Fowler VG, Benjamin DK Jr, Clark RH, Milkstone AM. Burden of invasive \textit{Staphylococcus aureus} infections in hospitalized infants. JAMA Pediatr. 2015;169(11):105—11.
4. Carey AJ, Duchon J, Della-Latta P, Salaman L. The epidemiology of methicillin-susceptible and methicillin-resistant \textit{Staphylococcus aureus} in a neonatal intensive care unit, 2000-2007. J Perinatol. 2010;30:135—9.
5. Dolapo O, Dhanireddy R, Talat AI. Trends of \textit{Staphylococcus aureus} bloodstream infections in a neonatal intensive care unit from 2000-2009. BMC Pediatr. 2014;14:121.
6. Ekkelenkamp MB, van der Bruggen T, van de Vijver DA, Wolfs TF, Bonten MJ. Bacteremic complications of intravascular catheters colonized with \textit{Staphylococcus aureus}. Clin Infect Dis. 2008;46:114—8.
7. Hakim H, Mylotte JM, Faden H. Mortality and mortality of staphylococcal bacteraemia in children. Ann J Infect Control. 2007;35:102—5.
8. Murdoch F, Daniel J, Morris AK, Czmak I, Bishop A, Glass E, Irniei JL. The Scottish enhanced \textit{Staphylococcus aureus} bacteraemia surveillance programme: the first 18 months of data in children. J Hosp Infect. 2017;97:127—32.
9. Le J, Darn Q, Tran T, Nguyen A, Adler-Shohet FC, Kim S, Schmidt K, Lieberman JM, Bradley JS. Epidemiology and hospital readmission associated with complications of \textit{Staphylococcus aureus} bacteraemia in children. JAMA Pediatr. 2015;35:1127—9.
10. Valente AM, Jain R, Scheurer M, Fowler VG Jr, Corey GR, Bengur AR, Sanders S, Li JS. Frequency of infective endocarditis among infants and children with \textit{Staphylococcus aureus} bacteraemia. Pediatrics. 2005;115:e15—9.
11. Kaufman O, Fairchild KD. Clinical microbiology of bacterial and fungal sepsis in very-low-birth-weight infants. Clin Microbiol Rev 2004;17:638–680, table of contents.
12. Verkaik NJ, Dauwalder O, Arnt H, Boubekeur I, de Vogel CP, Radoux C, Bes M, Vandenbroeck F, Tzir M, Hooijkaas H, Verbrugh HA, van Belkum A, Ettene J, Lina G, Ramdani-Bouguessa N, van Wamel WJ. Immunogenicity of toxins during \textit{Staphylococcus aureus} bacteraemia. Clin Infect Dis. 2010;50:601—8.
13. Rouard C, Bourgeois-Nicolaos N, Rahajamanana L, Romain O, Pouga L, Derouin V, De Luca D, Doucet-Populaire F. Evaluation of an ‘all-in-one’ seven-day whole-genome sequencing solution in the investigation of a \textit{Staphylococcus aureus} outbreak in a neonatal intensive care unit. J Hosp Infect. 2019;102:297–303.
14. Xu S, McCormick J. Staphylococcal superantigens in colonization and disease. Front Cell Infect Microbiol. 2012;2:52.
15. Regev-Yochay G, Rubinstein E, Barzilai A, Carmeli Y, Kuint J, Etienne J, Blech M, Smollen G, Maayan-Wetzler A, Leavitt A, Rahav G, Keller N. Methicillin-resistant Staphylococcus aureus in neonatal intensive care unit. Emerg Infect Dis. 2005;11:453–6.
16. Reich P, Boyle MG, Hogan PG, Johnson AJ, Eland AM, Warner BB, Burnham CA, Fritz SA. Emergence of community-associated methicillin-resistant Staphylococcus aureus strains in the neonatal intensive care unit: an infection prevention and patient safety challenge. Clin Microbiol Infect. 2016;22:645.e641–8.
17. Huang YC, Lien RI, Su LH, Chou YH, Lin TY. Successful control of methicillin-resistant Staphylococcus aureus in endemic neonatal intensive care units—a 7-year campaign. PLoS One. 2011;6:e23001.
18. Ramsing BG, Arpi M, Andersen EA, Knabe N, Mogensen D, Buhl D, Westh H, Ostergaard C. First outbreak with MRSA in a Danish neonatal intensive care unit: risk factors and control procedures. PLoS One. 2013;8:e66904.
19. Achermann Y, Seidl K, Kuster SP, Leimer N, Dürsich N, Ajdler-Schaffler E, Karrer S, Sepp N, Holzmann-Burgel A, Wolfensberger A, Leone A, Attertaz R, Zinkernagel AS, Sax H. Epidemiology of methicillin-susceptible Staphylococcus aureus in a neonatology Ward. Infect Control Hosp Epidemiol. 2015;36:1305–12.
20. Lin MF, Huang ML, Lai SH. Investigation of a pyoderma outbreak caused by methicillin-susceptible Staphylococcus aureus in a nursery for newborns. J Hosp Infect. 2004;57:38–43.
21. Gomez-Gonzalez C, Alba C, Otero JR, Sanz F, Chaves F. Long persistence of methicillin-susceptible strains of Staphylococcus aureus causing sepsis in a neonatal intensive care unit. J Clin Microbiol. 2007;45:2301–43.
22. Koningstein M, Groen L, Genaats-Peters K, Lutgens S, Rietveld A, Jira P, Kuytmanis J, de Greelf SC, Hermans M, Schneeberger PM. The use of typing methods and infection prevention measures to control a bullous impetigo outbreak on a neonatal ward. Antimicrob Resist Infect Control. 2012;1:37.
23. Leopold SR, Goering RV, Witten A, Harmen D, Mellmann A. Bacterial whole-genome sequencing revisited: portable, scalable, and standardized analysis for typing and detection of virulence and antibiotic resistance genes. J Clin Microbiol. 2014;52:2365–70.
24. Verkaik NJ, van Wamel W, van Belkum A. Immunotherapeutic approaches against Staphylococcus aureus. Immunotherapy. 2011;3:1063–73.
25. Borghesi A, Stronati M. Strategies for the prevention of hospital-acquired infections in the neonatal intensive care unit. J Hosp Infect. 2008;68:293–300.
26. Trevisanuto D, Arnolda G, Chien TD, Xuan NM, Le TA T, Kumara D, Lincetto O, Moccia L. Reducing neonatal infections in south and south central Vietnam: the views of healthcare providers. BMC Pediatr. 2013;13:51.
27. Ista E, van der Hoven B, Komelisic RF, van der Stare C, Vos MC, Boerma E, Helder OK. Effectiveness of insertion and maintenance bundles to prevent central-line-associated bloodstream infections in critically ill patients of all ages: a systematic review and meta-analysis. Lancet Infect Dis. 2016;16:724–34.
28. Goyal M, Javerliat F, Palmieri M, Mirande C, van Wamel W, Tavakol M, Verkaik NJ, van Belkum A. Genomic evolution of Staphylococcus aureus during artificial and natural colonization of the human nose. Front Microbiol. 2019;10:1525.

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