eP168

Molecular typing of Toxic shock syndrome toxin-1- and Enterotoxin A-producing Methicillin-sensitive Staphylococcus aureus isolates from a neonatal intensive care unit

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Introduction

Premature neonates are particularly susceptible to *Staphylococcus aureus* infections due to their immature immune system. In neonatal intensive care units (NICU)s, surgical procedures, the use of invasive devices and mechanical ventilations are factors which increase the risk for neonates to acquire *S. aureus* colonisation or infection. Furthermore healthcare-workers and parents serve as possible reservoirs and transmitters. Long-term studies reveal a heterogeneous population structure of *S. aureus* clones circulating in the respective NICU´s.

Outbreaks of *S. aureus* in NICUs are common and usually caused by methicillin resistant *S. aureus* strains (MRSA), including well-known hospital- and community-associated MRSA but also MRSA exhibiting special virulence factors like the Panton-Valentine-Leukozidin or Toxic-Shock-Syndrom-Toxin. Reports about outbreaks involving Methicillin-sensitive *S. aureus* are rare, mainly describing exfoliative toxins producing strains causing bullous impetigo in newborns.

Currently there is no consensus regarding the routine use of *S. aureus* screening surveillance in neonates, however some experts believe that surveillance especially in high risk group (as pre-term neonates) is essential.

In this study we investigated a cluster of colonisations and infections in a NICU over a one-year period, caused by Toxic shock syndrome toxin-1 (TSST-1) - and Enterotoxin A (EntA) -producing methicillin-sensitive *S. aureus* (MSSA) strain characterized by different typing methods.

Materials and Methods

Outbreak settings: In May 2012, three cases of serious infections (two neonates succumbed to toxic shock syndrome (TSS)) in a NICU of a tertiary care hospital in Berlin, prompting an outbreak investigation. On 30 May 2012 the Local Public Health Authority of Berlin was informed and the following investigation was supported by the Robert Koch Institute. The hospital is structured in four campuses located all over Berlin, with a total of 3,095 beds, about 12,700 employees and approximately 4,700 births yearly. The neonatal unit of the Mitte campus consists of two units: the intensive care unit (107i) and the intermediate intensive care unit (108i). The 107i unit consists of three rooms, for a total of nine beds (3/3/3). The 108i unit consists of six rooms for a total of 11 beds (4/3/1/1/1, of which 6 are also intensive-care beds) and an extra room for 1 mother and 1 neonate. The sickest and smallest infants are treated in the unit 107i. When neonate conditions improve, they can be either transferred to the 108i unit or be discharged.

Case definitions: Infected cases were defined as: a patient in 107i or 108i unit with signs of local or systemic infection, with an isolate of *S. aureus* characterised by *spa* type t021, positive for the *tst* gene and for the in-vitro production of TSST-1. Colonised cases were defined as: a patient in 107i or 108i ward with the respective clone. After the outbreak was recognised, medical registers were reviewed to identify retrospectively further cases.

Laboratory sampling and characterization of bacterial isolates: All *S. aureus* positive samples were sent to the NRC for Staphylococci and Enterococci. Antimicrobial susceptibilities, TSST-1- and EntA-production and presence of the respective toxin-genes were analyzed. Molecular typing was done by *spa*-typing and MLST. Relatedness of isolates was estimated by DNA microarray analysis (Alere StaphType) and *Sma*I-macrorestriction. Whole genome sequencing was performed using MiSeq technology. SNPs were identified by mapping paired-end sequencing reads against the genome sequence from a related CC30 isolate (MRSA 252). An alignment of SNPs in the genome s was used to reconstruct the isolates' phylogeny.

Conclusions

- Not only MRSA, but also MSSA strains cause severe life-threatening infections in neonates, especially if they are able to produce particular virulence factors.
- Parents and healthcare-workers serve as possible reservoirs and transmitters.
- We revealed a heterogeneous population structure of *S. aureus* clones circulating in the NICU.
- Discrimination of closely related strains even by a combination of sophisticated, molecular typing techniques might be limited and even misleading. Whole-genome sequencing provides new and more reliable insights into aspects of source-tracking, re-introduction and/or ongoing transmission of identical strain types at a local level.

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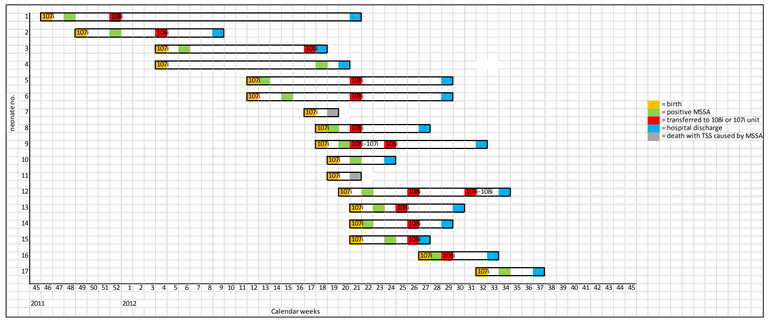
Results

Description of the outbreak

Fig. 1. Outbreak timeline of the neonates colonised or infected with MSSA in the hospital NICU, 11/2011-11/20120



Characterization of bacterial isolates

- MSSA exhibiting 56 different *spa*-types were identified
- 24 MSSA showed characteristics of the outbreak clone; *Sma*I-macrorestriction and DNA microarray analysis was performed for those isolates (figures 2 & 3).

Fig. 2. PFGE profiles of *Sma*I-digested genomic DNA of suspected outbreak isolates
(‡ isolates from parents; *isolates from staff)

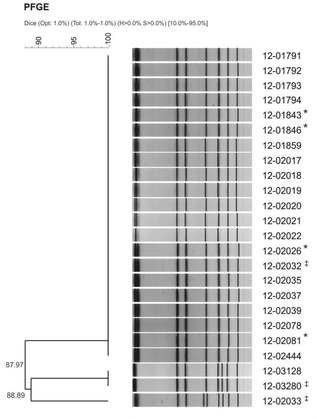
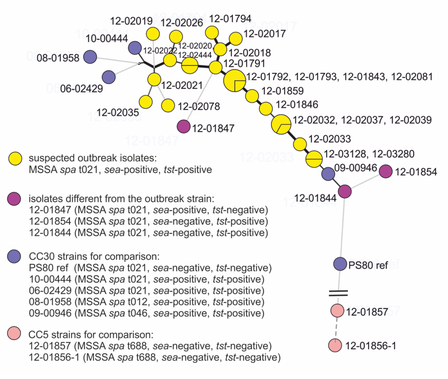


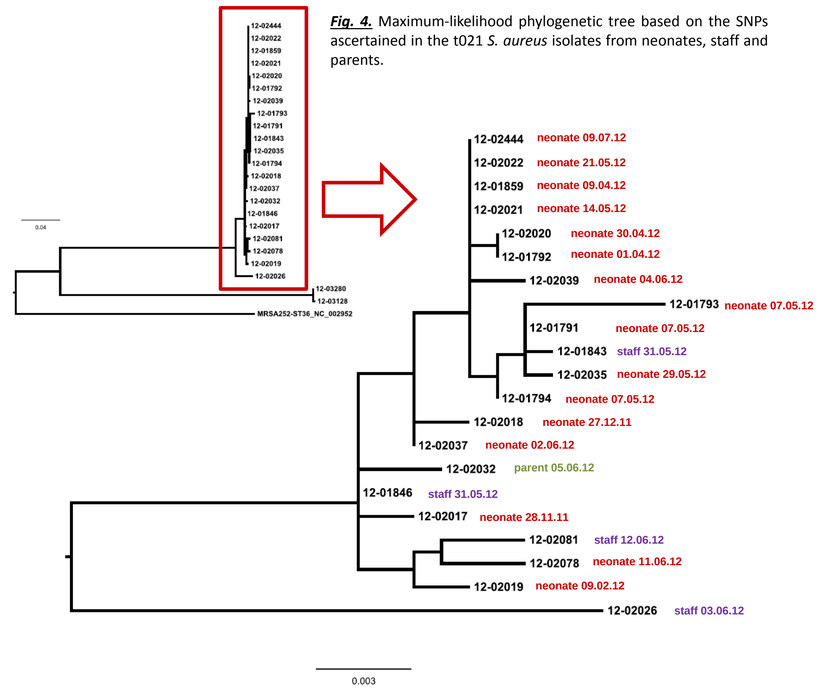
Fig. 3. Phylogenetic tree based on array data showing the relationships between the suspected outbreak isolates. Isolates different from the outbreak strain were added for comparison.



- Assignment of strains varied depending on the method applied.
- Based on *Sma*I-macrorestriction 21 isolates were closely related. Isolates from one neonate and two parents did not match with this cluster while non-related strains added for comparison clustered with the major clade (data not shown).
- Microarray analysis also revealed a close relationship of the 24 MSSA. If some of the isolates exhibiting the characteristics of the outbreak clone are not non-related could not be dissolved by this method.
- Whole genome sequencing identified epidemiological links among individual patients, between infants and their mothers, and between infants and staff members, which now enables us to proof several hypotheses on the transmission route of this clone.

- The scenario comprised two wards in a NICU of a tertiary care hospital in Berlin. Depending on an assumed index patient (which was identified after 3 cases of serious infections with **MSSA t021, TSST-1- and EntA-positive**; 2 babies died) this clone spreads within the two wards. Two further cases were identified retrospectively.
- In the following period from April to November 2012 162 MSSA were recovered from 213 neonates, 205 parents and 123 healthcare-workers (overall MSSA colonization rate 21.87%).
- 17 neonates, 4 staff members and 3 parents met the case definitions applied of infected or colonised cases. In figures 1 the 17 neonate cases are shown over the outbreak time period. Neonate couples 5-6, 8-9, 10-11 and 14-15 were twins. The first case (colonised case) was identified at week 48 in 2011, while the last case (infected case) was identified in the week 34 in 2012. Until week 45 of 2012, no more colonised or infected cases have been identified.
- The seventeen neonates were born between 15 November 2011 and 07 August 12 and all of them were transferred immediately to the neonatal intensive care unit 107i. Two neonates died after 18 and 20 days from birth, respectively. The other 15 neonates were either transferred to the 108i unit and then discharged or directly discharged from the 107i unit. These 15 neonates have been discharged from 37 to 196 days after birth, median 78.

Fig. 4. Maximum-likelihood phylogenetic tree based on the SNPs ascertained in the t021 *S. aureus* isolates from neonates, staff and parents.



Infection prevention and control measures

Enhanced hygiene; All parents had to wear masks when visiting positive neonates; All colonised staff members and neonate parents were decolonised with mupirocin; Positive staff members were excluded from the working environment until tested negative; Infected or colonised neonates received clindamycin; Very preterm infants received intravenous immunoglobulin as prophylaxis.