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Outbreak with methicillin resistant *Staphylococcus aureus* isolates lacking *mec* determinants on a neonatal ICU of a German hospital

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1 INTRODUCTION

Staphylococcus aureus MRLM (methicillin resistant lacking *mec*) strains are easily **misclassified as methicillin susceptible** (MSSA) based on the exclusive detection of *mec* genes or PBP2a. Hence, these isolates pose a **threat to public health** and represent a **diagnostic and therapeutic challenge**.

Recent studies demonstrated an association of the **MRLM phenotype** to **mutations in the *gdpP* gene** [1,2]. However, it is unknown how this MRLM phenotype is selected in the clinical environment.

AIM OF THE STUDY

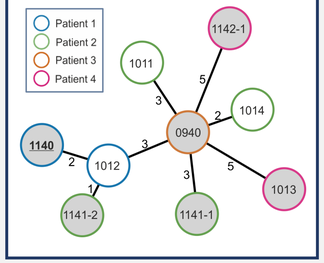
In this study, nine *S. aureus* strains (MRLM [n=6]; MSSA [n=3]), isolated from four infants during **routine nasal screening** at the **neonatal intensive care unit** (NICU) of a German hospital were investigated.

2 CLINICAL ISOLATES

Oxacillin and cefoxitin **minimal inhibitory concentrations** (MIC) were determined by broth microdilution according to EUCAST criteria [clinical breakpoints v.9.0]. The **presence of *mec* genes** was tested by PCR. ***Spa*-type**, **MLST** and **cg-MLST** complex-type (cgMLST CT) were deduced from **whole-genome sequences** (WGS).

Six isolates displayed **elevated MICs to oxacillin** (MIC ≥ 2 mg/L) **and cefoxitin** (MIC > 4 mg/L), but **lacked *mec* determinants** (MRLM).

FIGURE 1 **MINIMUM SPANNING TREE**



Based on WGS, the relatedness of isolates was analyzed and visualized in a **minimum spanning tree** based on 2249 loci of the *S. aureus* core and accessory genome (**FIGURE 1**).

All isolates were assigned to ***spa*-type t3338**, **ST7** and **CT20916**.

The strains differed from each other in a maximum of 8 of the 2249 genomic loci included in cgMLST analysis, indicating a **common origin of the isolates**.

3 RESULTS AND DISCUSSION

(A) OUTBREAK SETTING

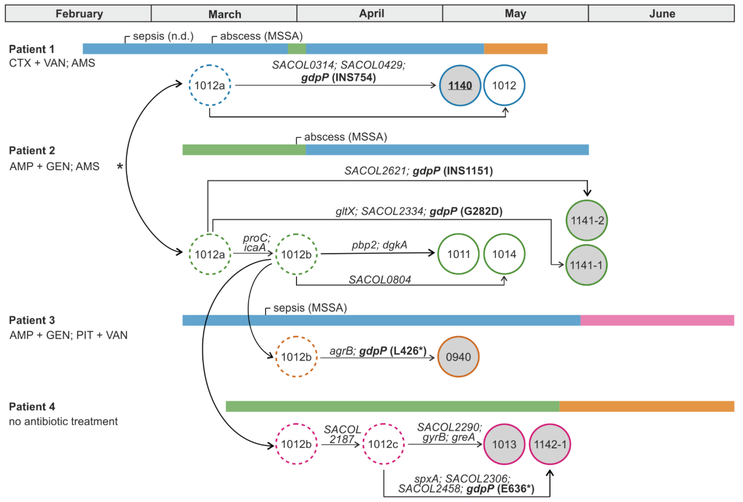
Based on **cgMLST data**, detailed SNP and indel analysis enabled the **reconstruction of possible transmission events** between patients by assuming the existence of non-isolated intermediate isolates (**FIGURE 2**).

The putative MSSA progenitor (1012a) was **spreading from an index patient**. The first transmission event probably occurred from P1 to P2 (or vice versa) since all strains except two (1141-1/2) that were isolated from P2-4 exhibit the same mutations [*proC* A45T; *icaA* G158S] (**FIGURE 2**).

Following the first **mutation event** in P2, the clone (1012b) **spread further** into P3 and P4, where it mutated in both patients independently.

Analysis of genomic data regarding the **MRLM phenotype**, revealed **various polymorphisms in the *gdpP* gene** in all but one isolate (1013) with elevated MIC values for CXI and OXA.

FIGURE 2 **CLINICAL TRANSMISSION SCENARIO**



MRLM isolates (n = 6) and MSSA isolates (n =3) have been collected from four patients during routine nasal screening. Horizontal arrows indicate possible mutation events of a strain. Curved arrows indicate possible transmission events between patients by assuming non-isolated intermediate isolates.

n.d., not determined; *transmission possible in both directions; CTX, Ceftazidim; VAN, Vancomycin; AMP, Ampicillin; AMS, Ampicillin/Sulbactam; GEN, Gentamycin; PIT, Piperacillin/Tazobactam

(B) SELECTION OF MRLM

In all patients independently, various **mutations in *gdpP*** occurred, suggesting a response to an existing **selective pressure**.

The therapy with **β-lactam antibiotics** would be an obvious assumption here. However, not all patients received **treatment** with the corresponding antibiotics (**FIGURE 2**).

The **selection of MRLMs** might be driven by something less specific, such as e.g., **disinfectants** used on the ward.

(C) MUTATION FREQUENCY

With respect to published mutation rates for *S. aureus* [3], an unexpected **high number of mutations** occurred within a short period of time (**FIGURE 2**). This might indicate for the spread of an isolate with **increased mutation frequency**.

This hypothesis is corroborated by a mutation in the **DNA repair gene *mutS*** in the outbreak clone [4]. All investigated isolates exhibit a *mutS* D768N amino acid substitution when compared to other ST7 isolates.

4 CONCLUSION

We describe an **outbreak involving MRLM on a NICU**. Our data indicate that initially an MSSA progenitor was transmitted between neonates. The strain seems to have acquired **resistance to β-lactams** in each infant independently, under the action of a hitherto **unknown selection pressure**, through different **mutations in *gdpP***.

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