Outbreak investigation of community associated methicillin-resistant Staphylococcus aureus in a primary and secondary school in Eastern Switzerland

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Abstract

Background: In 2018 the number of newly detected methicillin-resistant *Staphylococcus aureus* (MRSA) cases in children attending primary (age 6-12 years) and secondary school (age 13-16 years) nearly doubled compared to previous years.

Methods: This observation initiated an epidemiological outbreak investigation including a cross-sectional study to determine source of the outbreak, colonization frequency and to identify risk factors for transmission using a questionnaire and to end the outbreak.

Results: During the investigation, 49 individuals were detected with 57 corresponding isolates. Based on the case definition combined with whole genome sequencing, a core cluster was identified that shared common genetic features and a similar antimicrobial susceptibility pattern (efflux-mediated macrolide resistance, tetracycline susceptibility along with presence of Panton-Valentine leukocidin).

Conclusions: Epidemiologic evaluation identified a distinct school as a common risk factor. However, the source of the clustered infections within that school could not be further specified. No further cases could be detected after decolonization of infected and colonized children.

Introduction

Within the last 25 years infections with community associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) have increased all over the world. In Switzerland, overall MRSA detection rates decreased in recent years in adults, but increased in the younger population based on data from the Swiss antimicrobial resistance surveillance network (ANRESIS, http://www.anresis.ch) [1]. However, MRSA frequency in children admitted to hospitals is still low [2,3] and general screening is not recommended. Most children colonized with CA-MRSA remain asymptomatic. Symptomatic children mainly present with skin and soft tissue infections (SSTI) infections [4] but severe invasive infections are rarely seen. CA-MRSA infections are epidemic in some countries which may suggest, that CA-MRSA are more virulent and transmissible than hospital-associated MRSA strains [5]. CA-MRSA may frequently carry the cytotoxic toxin Panton-Valentin Leukocidin (PVL) as a virulence factor which is associated with severe manifestations of CA-MRSA [6]. Clusters of sequencing type 5 (ST5) PVL producing CA-MRSA strains in inpatient neonates [7], in school-children [8] and of different CA-MRSA strains have been previously described in Switzerland [9,10].

For many years, pulsed-field gel electrophoresis (PGFE) and multi-locus sequence typing (MLST) represented the standard MRSA typing techniques. Nowadays, because of next-generation sequencing (NGS) techniques, healthcare facilities are capable of tracing outbreaks with higher resolution than ever before. Whole-genome sequencing (WGS) as part of a surveillance system can be used to monitor pathogens of concern within a healthcare facility, a region or even countrywide. Furthermore, the use of core genome (cg) MLST allows a more in-depth view inside the evolution and spread of MRSA.

Because of detection of clustered infection of CA-MRSA in a tertiary children's hospital in Switzerland epidemiological and genomic outbreak investigations were initiated. Here we describe the epidemiological evaluation and characterization of the outbreak with CA-MRSA positive for PVL. The aims of the study were to identify the source and risk factors of transmission of CA-MRSA, to implement necessary measures to prevent transmissions and to end the outbreak.

Methods

Setting and background

As a result of continuous recording of newly detected multidrug-resistant bacteria doubled in comparison so previous years of CA-MRSA cases was observed in an 80 bed tertiary care children's hospital in the Eastern area of Switzerland during first semester of 2018. Furthermore, susceptibility test of most of the CA-MRSA cases revealed an efflux-mediated, non-inducible macrolide -streptogramin B antibiotic resistance pattern which further substantiate our observation. Epidemiological outbreak investigations were initiated which prompted the hypothesis that the outbreak was associated with a primary and secondary school in St. Gallen.

Microbial culture and MRSA detection

Copan eSwabs™ (Copan, Brescia, Italy) were used for swabbing and sent to the laboratory immediately after sampling. Enrichment broth (Brain heart infusion (BHI) with 6% NaCl, manufactured in house) was inoculated with 10 ml of the liquid medium and incubated overnight. Of the enriched broth 10 ml was inoculated onto a chromID chromogenic MRSA plate (bioMérieux, Marcy l'Etoile, France) with a WASP instrument (Copan, Brescia, Italy). After incubating the plates for 24 hours in the smart incubators of a WASPLab™ high-resolution images of media plates were inspected using the WASPLab™ WebApp software. Colonies, indicative for MRSA were identified by a software algorithm ("segregation") and sent to reading and picking for further workup [11].

Identification was done with a MALDI-ToF instrument using the BDAL 9.0 database (MALDI Biotyper Smart System, Bruker Daltonics, Bremen, Germany) with the colony transfer method ("direct smear"). Colonies, identified as *S. aureus* were further tested with a PBP2a (penicillin-binding protein 2a, which confers methicillin-resistance) lateral flow immunochromatography assay (Alere Clearview PBP2a Culture Colony Test, Alere Inc. Waltham MA, USA), which confirmed MRSA. A BD™ Phoenix instrument (Becton Dickinson, Sparks, MD, USA) with PMIC-88 cartridge (tailored for the susceptibility testing of staphylococci and other Gram-positive bacteria) was used for susceptibility testing, except vancomycin, which was tested with epsilometer (E-) test (bioMérieux, Marcy l'Etoile, France). Antimicrobial susceptibility testing (AST) data were interpreted according to respective EUCAST guidelines (version 8.0 or 9.0 in 2018 and 2019, AST data not shown). This procedure applies to all isolates processed by the Centre for Laboratory Medicine in St. Gallen. Five isolates were tested elsewhere (i.e. available as laboratory report, only) and the data could not be verified.

Whole Genome Sequencing

DNA was quantified using the Qubit dsDNA BR HS Assay Kit and Qubit fluorometers (Invitrogen, https://www.thermofisher.com). WGS was performed using Illumina MiSeq with the Nextera XTlibrary preparation kit (Illumina Inc., USA), according to the manufacturer's procedure. Trimming and assembly of raw reads was performed using the Velvet assembler of the SeqSphere software (Ridom, https://www.ridom.de, version 7.7.5 using *S.aureus* cgMLST v1.3 and *S.aureus* v1.1). The analysis included MLST, cgMLST (1`861 targets) and detecting resistance/virulence genes (*mecA*, macrolide resistance genes (*ermA, ermC, mphC, msrA*) and PVL (LukS-PV and LukF-PV)) by using SeqSphere. Coverage was at least 50-fold. We defined cgMLST clusters as groups of isolates with ≤5 different SNPs between neighboring isolates. To generate phylogenetic SNP trees, we used SeqSphere (Ridom; Münster, Germany) in the pairwise ignore missing values mode and an unweighted pair group method. The WGS data has been submitted to NCBI under the following submission number PRJNA738326. The genome assemblies were compared to a collection of 1861 MRSa isolates mostly originating from Switzerland collected at the University hospital Basel using the SeqSphere software.

Case definition

The following case definition was used to categorize MRSA cases. First, all culture positive MRSA cases were "**suspected cases**". Second, presence of the specific efflux-mediated macrolide resistance (phenotype) was assessed. Cases with an alternative antimicrobial susceptibility testing result were categorized as "**non-cluster cases**". "Suspected cases" having efflux-mediated macrolide resistance were "**probable cases**". In "probable cases" where strains were available, WGS was performed. Finally, if strains were ST5 using cgMLST, cases were "**confirmed cases**" and considered either as "core cluster" (≤5 single nucleotide polymorphisms (SNPs)) or "cluster" (>5 but <15 SNPs). All cases not belonging to ST5 or ST5 with >15 SNPs were again classified as "non-cluster cases" (see Figure 1).

Identification of risk factors

Risk factors for CA-MRSA were assessed using a questionnaire sent by email and/or mail to the families of confirmed and probable cases. This first questionnaire was sent in spring to summer 2019. Questions covered schooling (nursery, primary and secondary school), contact with the health-care systems (e.g., inpatient care), current, previous, or recurrent infections with MRSA or skin infections of the case and household contacts, profession, workplace, pets, leisure activities including sports, events, school or holiday camps or trips and attendance at the local children's festival held in June 2018.

Cross-sectional study

Given persistent transmission throughout 18 months (2018 - 2019), a cross-sectional study was initiated. Results of the epidemiological investigation and the first questionnaire supported the hypothesis that the outbreak was linked to a particular primary and secondary school. Accordingly, all students and all staff at the affected school with proven direct contact to the students (i.e. teachers, pre-schooling teachers)

were included in the study in November 2019. Clinical examination for skin lesions was performed in all students and staff. Swabs for MRSA (from axilla/inguinal region, nose/throat, wounds) were taken from all individuals with SSTI on clinical examination. These individuals also had to answer a second questionnaire, which was adapted from the previous one in summer 2018. Questions focused on school activities and leisure activities. Furthermore, every fifth student and staff without symptoms of CA-MRSA infection were randomly assigned to the cross-sectional study to receive swabbing and answer the questionnaire.

Statistics

Continuous variables were assessed by t tests or Mann-Whitney U tests as appropriate. For categorical values, comparison was done by Fisher's exact test. Missing data were not imputed. A two-sided p value < 0.05 was considered as statistically significant. Given the small number of each outcome, only the univariate but not the multivariable analyses are reported. Data analysis was performed with SAS (version 9.3, SAS Institute Inc., Cary, NC, USA).

Results

Outbreak description and epidemiological investigation

First cases of CA-MRSA were initially identified and treated at the local tertiary care children's hospital. A common denominator was quickly identified during clinical examination and case history taking: most cases were students attending a school within the same city. As a result of case accumulation within one school, the school medical service and the responsible health department were involved, and hygiene measures (intensified hand hygiene and disinfection of surfaces) initiated at the affected school in October 2018. To increase awareness of further CA-MRSA cases, pediatricians and family physicians in the city area were informed of an CA-MRSA outbreak and encouraged to take skin swabs if a typical clinical skin infections were observed and to decolonize in case CA-MRSA was detected and forward results to study team. Furthermore, parents of children from this school received an information letter in spring 2019 on CA-MRSA and the request to visit their family doctor if the child or themselves were symptomatic. Also, a media release was published in the regional newspaper. Physicians and parents were notified once more when the cross-sectional study took place ("awareness campaign").

Although the number of cases decreased during the winter 2018/2019, new cases continued to occur (see Figure 2). In the following, a first questionnaire was developed to identify other risk factors and determine joint venues and activities of cases. The questionnaire was distributed among 97% of confirmed and probable cases. Response rate was 65%, confirming that 23 (77%) cases were related to the school. Univariate analysis revealed no clustering regarding other risk factors of CA-MRSA than attendance of this school (data not shown). Affected children were not confined to a particular class, nor to any other shared venue or activity. Because no additional risk factors were identified beyond attendance at this

school, but additional infections were observed during the summer of 2019, a cross-sectional study was initiated (see below).

Clinical description of cases

CA-MRSA was identified in 49 individuals from January 2018 until June 2020. Eight cases had two isolates (total 57 different isolates). Two children were hospitalized. One adolescent was hospitalized because of osteomyelitis and in addition to intravenous antibiotic administration, surgical procedures and drainage were required. Based on the case definition, 27 cases were classified as confirmed (ST5 according to WGS), 6 as probable (not available for WGS) and 16 were non-cluster cases (9 cases were non-ST5 and 7 had a divergent antimicrobial susceptibility pattern). The non-cluster isolates either showed a MLS_B phenotype with resistance to clindamycin (i.e., constitutive, or inducible), did not have macrolide resistance or had non-ST5 on whole genome sequencing.

Most cases of the outbreak were children and had mild clinical symptoms of MRSA infection. Clinical characteristics of the 33 probable and confirmed cases are provided in Table 1. Association with the affected school was observed in 79% of cases (see Figure 1).

Table 1

Clinical characteristics of the outbreak. Adult cases included teachers and parents and a teacher's wife.

Characteristics	Children (n=28)	Adults (n=5)	All (n=33)
Mean age [IQR], years	10.5 [9-12]	37 [33 - 48]	11 [9 - 14]
Female sex (%)	13 (46)	2 (20)	15 (45)
Clinical diagnosis			
SSTI	22	5	27
Bacteremia and arthritis	1	0	1
Bursitis	1	0	1
Other	1	0	1
Colonization*	3	0	3
Association with school (%)	21 (75)	5 (100)	26 (79)
Confirmed cases, ST5 (%)	24 (86)	3 (60)	27 (82)

IQR = interquartile range, SSTI = skin and soft tissue infection, ST5 = sequencing type 5. *Colonized students were identified in the cross-sectional study.

Cross-sectional study

The cross-sectional study was performed between November 27-29, 2019, and 129 participants from the affected school were screened (104 students and 25 staff including teachers). SSTI were observed in 17 students (13%) but in no staff. MRSA infection was detected in 1.6% (2 students and no staff [p=0.64]) and MRSA colonisation in 4.7% (6 students and no staff [p=0.26]). Three of these six students had been symptomatic and identified as part of the MRSA cluster before but were not symptomatic at the time of the cross-sectional study. Skin infection was present both in participants with positive and negative MRSA swabs (p=0.35). WGS revealed ST5 in 87.5%. There was one non-cluster case (ST72). Using the modified questionnaire, we were again unable to identify risk factors for CA-MRSA colonisation (data not shown). CA-MRSA cases identified on the cross-sectional study underwent skin decolonization following local recommendations. Ongoing surveillance revealed recurrent infection in three cases but no new CA-MRSA cases (Figure 2).

Results of WGS

As a result of the outbreak investigation and the cross-sectional study, 39 cases (79%) were available for WGS (including seven individuals with two isolates) and 27 individual (69%) belonged to the core cluster (31 isolates whereof 4 had an additional follow-up isolate). Results from WGS included MLST typing (sequence type), detection of mecA (Methicillin resistance gene), the macrolide resistance genes (*ermA, ermC, mphC, msrA, vga, vgbB*), PVL genes (LukS-PV/LukF-PV) and the case definition (Table 2).

35 CA-MRSA isolates belonged to ST5 and differed by a maximum of 259 SNPs. The outbreak cluster consisted of 31 isolates with a maximum of 9 SNPs (Figure 3). The number of SNPs increased over time (Figure 4). Within the sequenced ST5 isolates were five pairs from the same individual but 2 to 23 months apart, which also showed differences of 0,1, 2, 4, and 8 SNPs compared to the closest ST5 strain. This suggests evolution over time during this event. The methicillin and macrolide resistance genes (mecA, ermA, ermC, mphC, msrA) matched to their respective phenotypes. The macrolide resistance genes of the core cluster were a combination of the mphC and msrA genes, except for one isolate (SGMRSA37) that only harbored mrsA. Besides the core cluster, several isolates harboring the MLS_B genes ermA and ermC were also discovered.

Table 2

Results of whole-genome sequencing of CA-MRSA isolates (N=46)

Sample ID	Sampling date	ST	mecA	macrolide resistance genes	PVL gene (Luk F/S)	Case definition	Source
SGMRSA1	09.02.18	22	+	-	-	non-cluster isolate	OI
SGMRSA1_2	09.02.18	22	+	-	-	non-cluster isolate	OI
SGMRSA2	20.04.18	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA3	06.02.18	8	+	-	+	non-cluster isolate	OI
SGMRSA3_2	12.02.18	8	+	-	+	non-cluster isolate	OI
SGMRSA4	19.04.18	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA5	18.04.18	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA6	12.04.18	30	+	ermA	-	non-cluster isolate	OI
SGMRSA7	09.04.18	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA8	29.03.18	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA8_2	26.11.19	5	+	mphC, msrA	+	core cluster isolate	CS
SGMRSA9	22.03.18	22	+	ermC	+	non-cluster isolate	OI
SGMRSA10	23.02.18	22	+	-	-	non-cluster isolate	OI
SGMRSA11	20.04.18	5	+	mphC, msrA	+	core cluster isolate	OI

SGMRSA11_2	03.04.19	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA12	10.03.18	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA13	06.05.18	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA14	17.02.18	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA14_2	26.11.19	5	+	mphC, msrA	+	core cluster isolate	CS
SGMRSA15	06.06.18	5	+	-	-	non-cluster isolate	OI
SGMRSA16	05.06.18	8	+	-	-	non-cluster isolate	OI
SGMRSA17	02.07.18	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA18	03.07.18	5	+	-	+	non-cluster isolate	OI
SGMRSA19	05.07.18	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA20	14.11.18	6584	+	-	+	non-cluster isolate	OI
SGMRSA21	03.09.18	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA22	19.09.18	5	+	ermA	-	non-cluster isolate	OI
SGMRSA23	24.09.18	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA24	14.11.18	5	+	mphC, msrA	+	core cluster	OI

						isolate	
SGMRSA25	02.08.18	1472	+	mphC, msrA	+	non-cluster isolate	OI
SGMRSA26	04.06.19	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA27	14.01.19	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA28	17.11.18	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA29	28.06.19	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA29_2	27.11.19	5	+	mphC, msrA	+	core cluster isolate	CS
SGMRSA30	05.07.19	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA31	13.08.18	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA32	30.06.18	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA32_2	25.05.20	5	+	mphC, msrA	+	cluster isolate	OI
SGMRSA33	25.11.19	5	+	mphC, msrA	+	core cluster isolate	CS
SGMRSA34	26.11.19	5	+	mphC, msrA	+	core cluster isolate	CS
SGMRSA35	26.11.19	72	+	mphC, msrA	-	non-cluster isolate	CS
SGMRSA36	25.05.20	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA37	27.11.19	5	+	-	+	core cluster	CS

						isolate	
SGMRSA38	27.07.20	5	+	mphC, msrA	+	core cluster isolate	OI
SGMRSA39	20.03.19	5	+	mphC, msrA	+	core cluster isolate	OI

+: presence of mecA, LukS/F; -: absence of mecA, LukS/F, Sequence Type: according to *S.aureus* MLST; PVL: Panton-Valentine Leukocidin; Extension _2 means duplicate or follow-up isolate (depending on the date of isolation) of a given patient

SNP tree generated by SeqSphere using the pairwise ignore missing values mode (46 samples with 28657 columns) and an unweighted pair group method. Numbers refers to sample ID (see table 2). Numbers close to connecting lines between the isolates represent the SNPs. The different connecting lines reflect the relatedness between the respective isolates. Figure shows 27 confirmed cases plus 4 duplicates. SGMRSA32_2 with 9 SNPs difference is not shown.

Isolates are stratified according to month of isolation. The number of SNPs observed with respect to the first isolate (SGMRSA14) is given. Note, that isolates obtained in November 2019 were from the cross-sectional study. Colors code the numbers of SNPs, with dark green for 0 SNPs. Increasing SNPs are colored from yellow to red.

Discussion

This epidemiological and microbiological investigation of 49 children and adults with a newly detected CAMRSA identified 33 cases that belonged to the outbreak. Molecular typing revealed a cluster with ST5 and a common efflux-mediated, non-inducible macrolide-lincosamid-streptogramin B antibiotic resistance pattern. Epidemiologic investigation found that in 79% of cases, a local elementary and secondary school (one campus) were the main sites of transmission. Following the implementation of interventions in the school and surrounding community as well as the conduct of a cross-sectional study with subsequent decolonization of participants in whom CA-MRSA could be found and no further cases occurred.

Although several CA-MRSA clusters have been described in Switzerland before, this is - to the best of our knowledge - the largest cluster of symptomatic children reported to date. A cluster of ST5 PVL producing CA-MRSA strains has been previously described in Switzerland [7]. However, this cluster included seven inpatient neonates with a phenotypically different MRSA with respect to the antimicrobial resistance. In a surveillance report on 58 CA-MRSA cases from Western Switzerland, 13 distinct transmission clusters within 26 cases were detected over 3-year period [9]. About one fifth were children under 10 years of age and about two thirds of the isolates produced the PVL toxin. Finally a PVL-producing cluster of methicillin-susceptible *Staphylococcus aureus* within 10 of 22 schoolchildren and one of two teachers has been reported in Western Switzerland [8]. Six children were symptomatic with skin infections but

none of the strains involved were MRSA. Similar to the cluster described here, no further cases occurred after implementation of treatment and decolonization.

Molecular microbiological methods (e.g. WGS) enabled clear delimitation and description of this CAMRSA ST5 cluster, providing important information for evaluating whether, where and for how long preventive measures are needed. This is highly useful information for managing an outbreak. Still, although we surveyed cases and families on risk factors by questionnaires and conducted a cross-sectional study in the affected school, the key important insight on the common source of this CA-MRSA cluster remained unknown.

From the discussions during the investigations with the school representatives, the school medical service and the responsible health department, a hypothesis remains, which is supported by the WGS results. Initially, from February to November 2018, nine strains were identified by WGS with no difference in cgMLST (i.e. zero SNPs). These findings suggest an initial transmission event occurred in spring 2018.

We can only speculate about the exact timing and circumstances of this event. But it may have been in the context of a regional children's festival in the spring of 2018, which takes place every four years. During rehearsals as part of the preparations, clothes worn directly on the skin were immediately changed between the children without being washed. Unfortunately, the questionnaire did not confirm this hypothesis since all children (with and without CA-MRSA) attended this event. However, given most children in the region participate, this is not unexpected. Further and less frequent transmissions during the following years must have taken part during school and leisure activities since CA-MRSA is transmitted by direct contact.

Consistent with the epidemic curve (Fig. 2), the isolates had increasing numbers of SNPs over time compared to the first isolate in February 2018. While the actual magnitude of previous clusters reported from the literature remains unclear, we here provide evidence of a spatially and temporally restricted ST5 CA-MRSA PVL producing cluster. Both, a canton-wide request to all laboratories and analysis of a database with isolates from all over Switzerland (data not shown) revealed that this was a distinct cluster as characterized by WGS.

Finally, we identified 4.7% CA-MRSA carriage on a school-based cross-sectional study during an outbreak and CA-MRSA infection in 1.6%. Data on the frequency of CA-MRSA carriage in the healthy population is not readily available since antimicrobial resistance testing according to the national antimicrobial resistance surveillance network ANRESIS is based on clinical isolates and not on swabs of healthy individuals. Thus, the rate of colonisation in healthy students with CA-MRSA in Switzerland is unknown. There are data from a single-center cross-sectional study in 340 hospitalized children with no MRSA detection [3]. Asymptomatic colonization was found in 0.1% of pre-clinical students in Warsaw, Poland [12]. Higher rates have been observed in nursing homes (14.5%-38%) [13, 14], intravenous drug users (8.7%) [15] and in persons with contact with life stock (6.7%) [16]. In a worldwide study comprising of > 1000 surgeons only 2% were colonized with MRSA [17] and 4.6% of health-care workers on meta-analysis [18]. We therefore consider the CA-MRSA carriage rate of 4.7% in healthy individuals to be above what

would be expected in our setting. The identification of a single non-ST5 isolate on cross-sectional study (0.78%) that likely represents baseline carriage further strengthens our hypothesis. Overall, and although the ST5 strain produced PVL, the clinical phenotypes in the children were benign and non-invasive, except for one case with bacteremia and osteomyelitis.

Our study has several limitations: Although we compared out data with a nationwide strain collection, we still have only a limited view on the “big picture”. We can only speculate that in the majority of CA-MRSA-infections (i.e. SSTI) isolates are not being characterized by cgMLST. Along with this, nation-wide analysis is likely biased towards isolates from the hospital setting. In addition, we did not conduct the cross-sectional study in a control school without clustering of cases. Therefore, we are not able to compare the results with the MRSA carrier rate in asymptomatic school children. Finally, while we could not determine the exact start of the cluster, transmission chains or independent predictors for the cluster. However, the observed microevolution (i.e. accumulation of SNPs) suggests an initial transmission event with accumulated SNPs over time, reflecting a molecular clock, driving genetic diversity.

There are several points that substantiate our findings. First, we did not only focus on the initial cluster but followed school classes longitudinally followed for over 2 years. Since single cases of CAMRSA SSTI still occurred at that time, a rigid cross-sectional study was conducted. This allowed us not only to describe the cluster itself, but to analyze microevolution over time. Second, since the number of SNPs within the cluster was low as expected, we performed WGS of randomly chosen MRSA isolates over time to diversify the molecular data. This was done for three isolates, two of the cluster and one isolate of different ST and phenotype. Third, to get the broader overview, we compared the sequences obtained during this study to a collection of 1861 MRSA isolates primarily from Switzerland.

Conclusion

We identified and characterized a school outbreak of ST5 PVL CA-MRSA in 33 individuals by epidemiological investigation combined with WGS. As detailed risk assessment and a cross-sectional case-control study revealed no other source of transmission than a local school, therefore transmission likely occurred during school activities. Our data suggest an initial transmission event that could have been the local children’s festival. After the implementation of hygienic measures, information of parents and local physicians and the treatment and decolonization of all infected and colonized CA-MRSA carriers no further cases were detected during continued surveillance. A combined approach of epidemiological and microbiological methods is successful to terminate an outbreak of CA-MRSA.

Declarations

Ethics approval and consent to participate

No formal informed consent was obtained as this study involved research with anonymized health-related data. The study was commissioned and supported by the responsible health authorities.

Consent for publication

Not applicable

Availability of data and material

The datasets generated and/or analyzed during the current study are currently not publicly available. Deidentified participant data might be available on reasonable request by email to the corresponding author. The WGS data has been submitted to NCBI under the following submission number PRJNA738326.

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Authors contributions

CRK and ON initiated and designed the study with the support of MS, CB, and DZ. CRK and ON supervised the study. FW and CK performed the epidemiological investigation including the development of questionnaires and conducted the cross-sectional study with support of MS, SM and AW. FW and CK drafted the first version of the manuscript with support of ON, SaS and WA. ON and SaS were responsible for the microbiology work and analysis of NGS data with the support of AE and TR. All authors contributed to data interpretation and revised and approved the manuscript for intellectual content. All authors agreed to the final version including submission for publication and accept responsibility for this work.

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Competing Interests

The authors declare that they have no competing interests.

References

1. Olearo F, Albrich WC, Vernaz N, Harbarth S, Kronenberg A, Swiss Centre For Antibiotic Resistance Anresis. Staphylococcus aureus and methicillin resistance in Switzerland: regional differences and trends from 2004 to 2014. Swiss Med Wkly **2016**; 146:w14339.
2. Heininger U, Datta F, Gervaix A, et al. Prevalence of nasal colonization with methicillin-resistant Staphylococcus aureus (MRSA) in children a multicenter cross-sectional study. Pediatr Infect Dis J **2007**; 26:544–546.
3. Johnson K, Frei R, Heininger U. Prospective, cross-sectional study on MSSA and MRSA colonisation in hospitalised children.Eur J Pediatr **2015**; 174:1255–1262.
4. Herold BC, Immergluck LC, Maranan MC, et al.Community-acquired methicillin-resistant Staphylococcus aureus in children with no identified predisposing risk. JAMA **1998**; 279:593–598.
5. DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated meticillin-resistant Staphylococcus aureus. Lancet **2010**; 375:1557–1568.
6. Saeed K, Gould I, Esposito S, et al. Panton–Valentine leukocidin-positive Staphylococcus aureus: a position statement from the International Society of Chemotherapy. International Journal of Antimicrobial Agents **2018**; 51:16–25.
7. Sax H, Posfay-Barbe K, Harbarth S, et al. Control of a cluster of community-associated, methicillin-resistant Staphylococcus aureus in neonatology. Journal of Hospital Infection **2006**; 63:93–100.
8. Boubaker K, Diebold P, Blanc DS, et al. Panton-Valentine Leukocidin and Staphyloccoccal Skin Infections in Schoolchildren - Volume 10, Number 1—January 2004 - Emerging Infectious Diseases journal - CDC. Available at: https://wwwnc.cdc.gov/eid/article/10/1/03-0144_article. Accessed 15 November 2020.
9. Aramburu C, Harbarth S, Liassine N, et al. Community-acquired methicillin-resistant Staphylococcus aureus in Switzerland: first surveillance report.Euro Surveill **2006**; 11:42–43.
10. Meyer Sauteur PM, Leimer N, Chan PK, et al.Methicillin-resistant Staphylococcus aureus eradication in young infants should include the diaper area and treat housing contacts.Acta Paediatrica **2015**; 104:e581–e584.
11. Faron ML, Buchan BW, Vismara C, et al.Automated Scoring of Chromogenic Media for Detection of Methicillin-Resistant Staphylococcus aureus by Use of WASPLab Image Analysis Software. J Clin Microbiol **2016**; 54:620–624.
12. Szymanek-Majchrzak K, Kosiński J, Żak K, Sułek K, Młynarczyk A, Młynarczyk G. Prevalence of methicillin resistant and mupirocin-resistant Staphylococcus aureus strains among medical students of Medical University in Warsaw. Przegl Epidemiol **2019**; :39–48.
13. Moschou A, Maraki S, Giormezis N, et al. Prevalence and molecular epidemiology of Staphylococcus aureus nasal colonization in four nursing home residents in Crete, Greece. Journal of Infection and Chemotherapy **2020**; 26:199–204.
14. March A, Aschbacher R, Dhanji H, et al. Colonization of residents and staff of a long-term-care facility and adjacent acute-care hospital geriatric unit by multiresistant bacteria. Clinical Microbiology and Infection **2010**; 16:934–944.

15. Packer S, Pichon B, Thompson S, et al. Clonal expansion of community-associated meticillin-resistant Staphylococcus aureus (MRSA) in people who inject drugs (PWID): prevalence, risk factors and molecular epidemiology, Bristol, United Kingdom, 2012 to 2017. Eurosurveillance **2019**; 24. Available at: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2019.24.13.1800124. Accessed 29 April 2021.
16. Neradova K, Jakubu V, Pomorska K, Zemlickova H. Methicillin-resistant Staphylococcus aureus in veterinary professionals in 2017 in the Czech Republic.BMC Vet Res **2020**; 16:4.
17. Morgenstern M, Erichsen C, Hackl S, et al.Antibiotic Resistance of Commensal Staphylococcus aureus and Coagulase-Negative Staphylococci in an International Cohort of Surgeons: A Prospective Point-Prevalence Study. PLoS ONE **2016**; 11:e0148437.
18. Albrich WC, Habarth S. Health-care workers: Source, vector, or victim of MRSA?Lancet Infect Dis **2008**; 8(5):289-301.

Figures

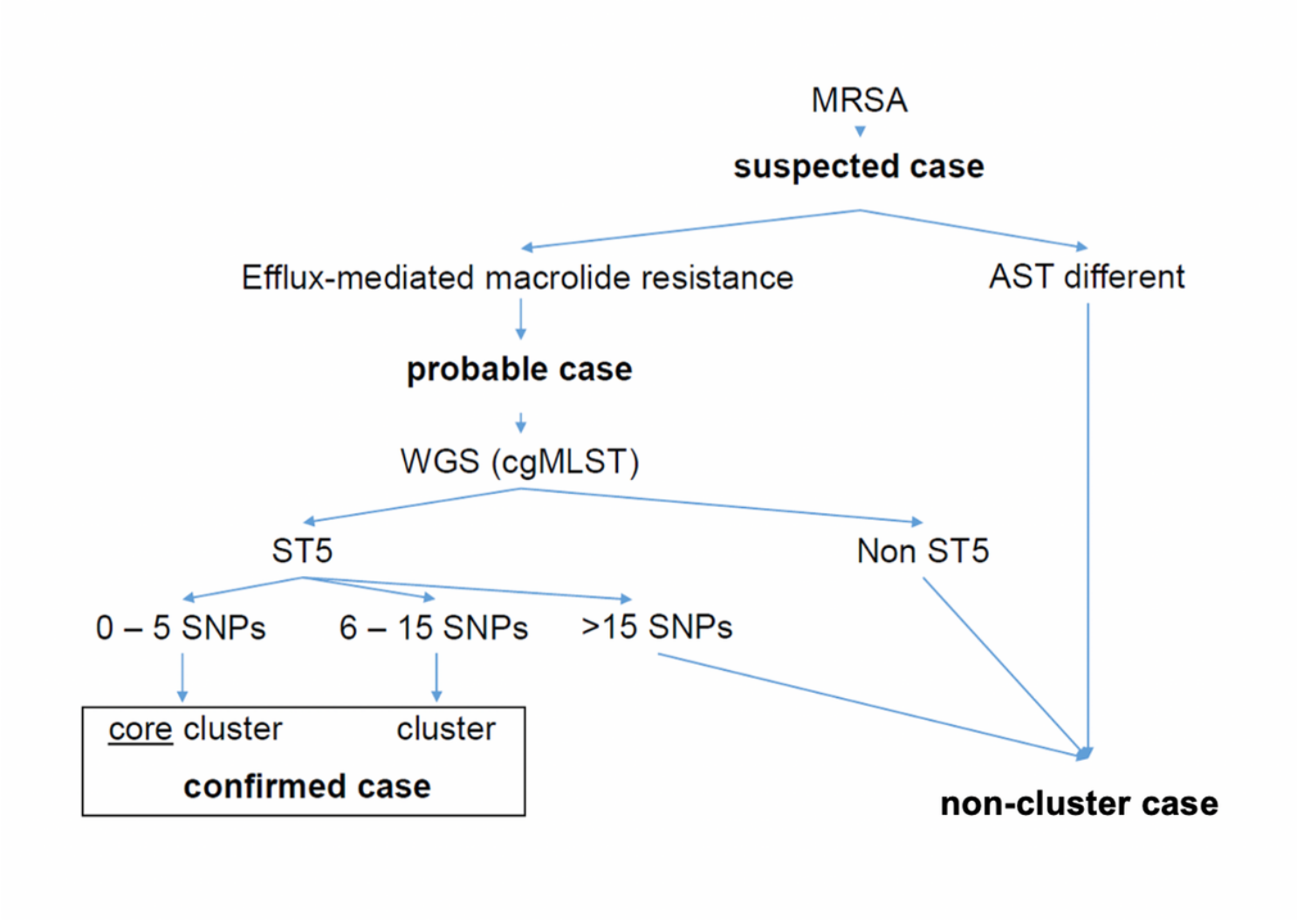

Figure 1

Case definitions used in this study

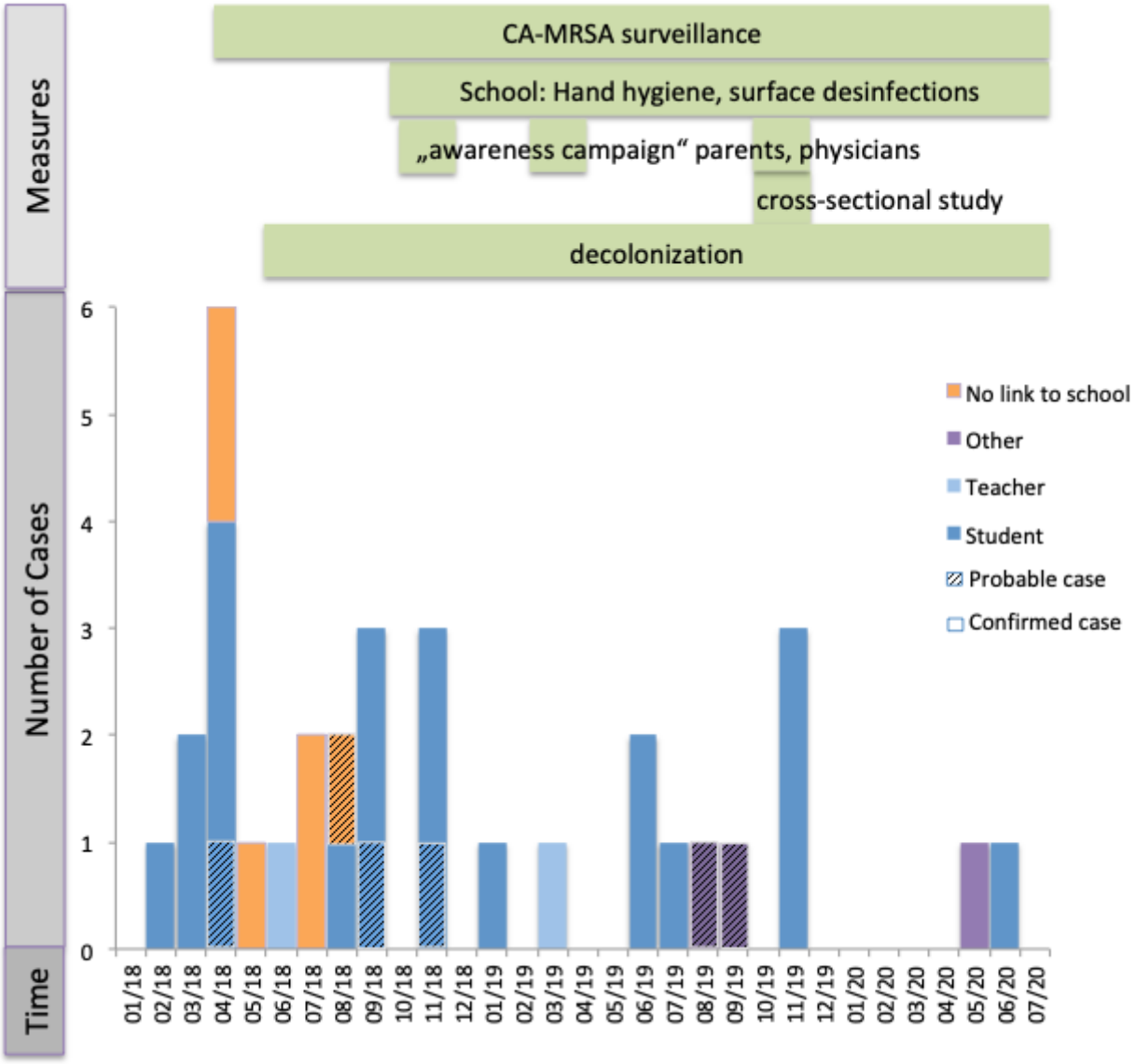

Figure 2

Epidemic curve of confirmed and probable cases with measures initiated during outbreak investigation (n=33)

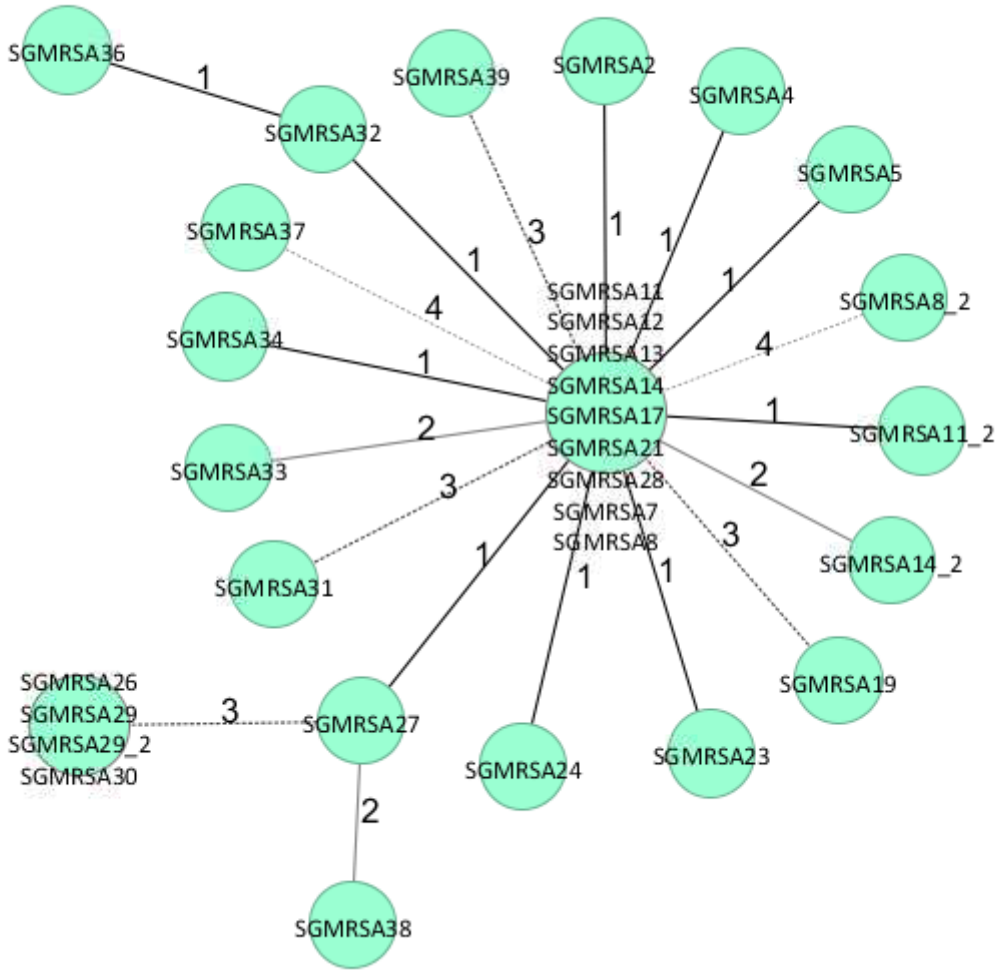

Figure 3

Phylogenetic single nucleotide polymorphisms (SNP) tree of the core cluster (N=31)

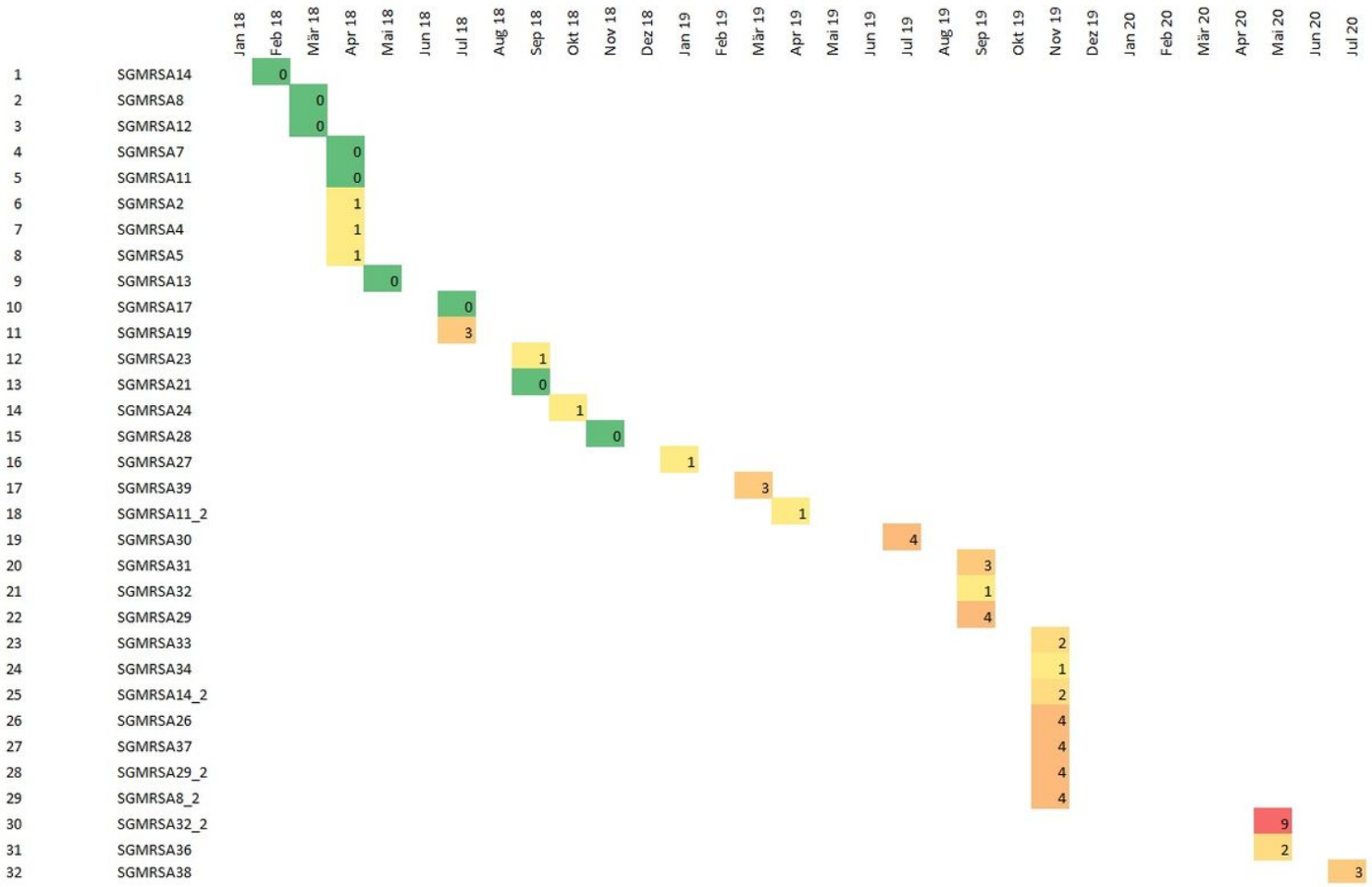

Figure 4

Graphical representation of micro-evolution within the ST5 cluster over time.