

Methicillin-Resistant Staphylococcus Aureus Transmission and Hospital-Acquired Bacteremia in a Neonatal Intensive Care Unit in Greece

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Abstract

The epidemiology of methicillin-resistant *S. aureus* (MRSA) colonization and infections in a 30-bed, level III university-affiliated neonatal intensive care unit was retrospectively investigated (2014-2018). Virulence, resistance genes and clonality of 46 isolates were determined by PCRs and MLST. Of 1538 neonates, 77 (5%) had a positive culture for MRSA; four bacteremias occurred. One major clone was identified, ST225 (23/40, 58%), imported from the same maternity hospital. Another clone, ST217, was predominant (4/6) among colonized health care workers. Four isolates classified as ST80 were PVL-positive, four *tst*-positive, and two *etb*-positive. Strengthening of infection control measures with emphasis on hand hygiene was applied.

Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of healthcare associated infections worldwide. Neonates constitute a special group in which MRSA infections can pose a significant burden of morbidity and mortality. Neonatal Intensive Care Units (NICUs) are an important reservoir of introduction and transmission of various MRSA clones among parents, health care workers (HCWs) and neonates [1]. The acquisition rate of initially non-colonized neonates admitted in a NICU has been reported to be around 6% with a median time of acquisition of nine days [2]. Around 20% of colonized neonates will develop an infection with a mortality rate 3-28% [3].

The majority of MRSA infections worldwide are due to a limited number of clonal lineages. The most common MLST types in neonatal wards are ST1, 5, 8 and 22 [3]. In Greece, the ST80-SCC*medV* prevailed in the community over 12 years (61.6% in total and 88.8% in CA-MRSA), and ST239-III in hospitals (22.5% in total and 60.8% in HA-MRSA) [4].

An observational study was performed to assess the prevalence of MRSA carriage and invasive infections in a 30-bed level III university-affiliated NICU at the P. & A. Kyriakou Children's Hospital between January 1st, 2014 and December 31st, 2018. The NICU admits a large proportion of outborn infants, prematures included as well as neonates with malformations or complex conditions requiring surgical care.

Surveillance and clinical care cultures of neonates that grew MRSA were retrospectively reviewed for the period 2014-2018, after a case outbreak of MRSA bacteremias during the last months of 2017. Following this outbreak, all HCWs were investigated for MRSA carriage too, with repeated nasal cultures.

NICU-acquired MRSA colonization or infection is defined when a positive surveillance/clinical care culture is associated with a minimum stay of three days in the NICU of our hospital [5]. If admission culture or culture within two days of admission grows MRSA, then NICU-imported MRSA is considered.

Identification and susceptibility testing of *S. aureus* was performed with conventional methods according to EUCAST guidelines [6]. All isolates with a cefoxitin inhibition zone of ≤ 21 mm were tested with a latex

agglutination test (Slidex MRSA Detection; bioMérieux®) for the presence of penicillin-binding protein 2a, and with PCR for *mecA* and *mecC* [7].

Amplification of genes encoding Panton-Valentine Leukocidin (PVL, *lukS/lukF-PV*), exfoliative toxins (*eta*, *etb*), toxic shock syndrome toxin (*tst*), and the resistance gene *fusB* (fusidic acid) was performed by PCRs among 46 representative strains (40 from patients and six from HCWs), as described [7-9].

The 46 selected *S. aureus* strains were characterized by MLST (<http://mlst.net>) [10]. Results were analyzed by the application of eBURST algorithm. Clonal complexes were defined by using the default setting.

During the study period 1538 neonates accounting for 26673 patient days were admitted to the NICU with a median length of stay 17 days (IQR: 11 to 56 days). All patients were either transferred from another NICU or from maternity hospitals (MHs). In total, 77 neonates (5%) had a positive culture for MRSA. Fifty-one (66%) were boys. Twenty three of 77 (29.9%) were NICU-acquired and 54/77 (70.1%) were imported cases. The hospitalization period before colonization ranged from 4 to 131 days (median 28, IQR: 11-65days). Four colonized boys (5.2%) developed MRSA bacteremia. Teicoplanin was successfully administered to all for seven days. Most isolates were multi-resistant, with higher resistance percentages observed against kanamycin (71%), macrolides (49%), lincosamides (47%) and ciprofloxacin (39%). All were susceptible to teicoplanin and vancomycin. All 37 HCWs were also tested and six among them were found positive for MRSA. All 46 (40 obtained from neonates and six from HCWs) molecularly analyzed strains were *mecA*-positive. Ten fusidic acid-resistant isolates were *fusB*-positive. One major clone was identified, ST225, among 40 tested neonatal strains (23/40, 58%). Of these, 14/23 were imported from the same MH. Another clone, ST217 comprising seven isolates, was predominant among HCWs (4/6) found to be colonized during screening performed on January 2018. ST30 and ST80 with seven and four strains respectively, were also identified. NICU-acquired bacteremia occurred in four neonates on Nov16, Aug17, Oct17 and Jan18 due to ST217 and ST225, three and one cases, respectively. Four isolates classified as ST80 were PVL-positive. Four additional strains carried *tst* (10%), belonging to ST30 and ST225 (two strains each), and two *etb* (5%, ST225). Decolonization treatment with nasal mupirocin and chlorhexidine baths was initiated and successfully performed in all colonized neonates and HCWs and all were negative for staphylococcal carriage upon subsequent screening. The implicated MH was notified for the problem and strengthening of infection control measures with emphasis on hand hygiene was applied.

In this study we describe the epidemiology of MRSA colonization and infection in a Greek referral NICU as well as the molecular characteristics of the implicated strains. To our knowledge, this is the first study concerning molecular characteristics of MRSA strains in a NICU in Greece. Four clones (ST225, ST217, ST30, ST80) were found colonizing the neonates and of these, two (ST225 and ST80) were implicated in bacteremias. The sources of this variable population were located in both within and outside the hospital.

In our NICU, colonization rate was 5%. In a study from USA among 3536 neonates from 2007 to 2011, 2% had a culture grow MRSA [3]. A rate of 5,2% MRSA colonization among 536 neonates was recently

reported from China for the period 2015-2016 [11]. Similar findings come from USA, where 3,9% of 3700 neonates from a single NICU were colonized [12]. In a recent multicenter epidemiological study conducted with the participation of 16 Greek NICUs for the period 2012-2015, *S. aureus* accounted for only two late onset septic episodes among 459 in total and the implicated strains were MSSA [13]. In the current study 5,2% of colonized neonates developed MRSA bacteremia. This rate is lower than that reported by Dong et al, where one out of five MRSA colonized neonates may develop bacteremia [2].

Regarding the detected clones, ST225 was the most common healthcare associated (imported) clone introduced by a sole MH of Athens, followed by ST217 and ST30. The MH was immediately notified and infection control bundles such as cohorting-isolation of colonized newborns, use of contact precautions, reinforcement of hand hygiene to personnel, education and training of new staff and outset of active surveillance shortly after the notified outbreak were imposed. In a report from Greece, among 194 erythromycin-resistant MRSA isolates, phylogenetic analysis showed that ST225, which belongs to CC5, was the most prevalent clone, accounting for 137 MRSA isolates. Sequencing of two isolates revealed a plethora of toxin genes of the enterotoxin family increasing its pathogenicity [15]. ST225-MRSA-II is a single locus variant (SLV) of the ST5-MRSA-II pandemic CC5 strain [16]. Isolates of ST5/ST225-MRSA-II have been recovered in Austria, Croatia, Hong-Kong (China), Hungary, Japan, Portugal, Taiwan, UK and USA [16]. In our study, 20/23 ST225 isolates were erythromycin and clindamycin resistant too, exhibiting a MDR phenotype. *erm(A)* and the aminoglycoside resistance gene *aadD* are commonly present in ST5/ST225-MRSA-II isolates [16]. Only two of ST225 isolates were gentamicin-resistant in this study.

In three out of four neonates with bacteremia, ST217 was implicated. This clone was NICU-acquired and was isolated from 4/6 HCWs. Contact with HCWs is an important factor for colonization of neonates [14]. Although we had limited data on HCW MRSA colonization prevalence during the study period, we identified HCWs as the main bacterial reservoir for institutional transmission and subsequent bacteremia. Very few ST217 strains are currently found in the MLST database (<http://saureus.mlst.net>), and data on such strains are scant in the literature. In particular, ST217-MRSA-IV was one of the dominant MRSA lineages isolated from patients in a hospital in Switzerland and Italy and was detected in food samples of animal origin in Spain [17]. ST217 is a single-locus variant of EMRSA-15 and might have been evolved from the ST22-MRSA-IV clone. The presence of ST217 was documented in India in 2012 [18].

The ST80 clone was reported for the first time in Greece in 2003, which accounted for 9.3% (11/118) of all MRSA strains isolated [19]. Since then, several studies reporting different percentages of MRSA-ST80 among all MRSA isolates have been published, including a 12 year survey (2001 to 2012) from geographically diverse areas of the country which showed the epidemic proportion of this clone in the community, accounting for 2838 isolates (88.8% CA-MRSA and 11.2% HA-MRSA isolates). This clone is predominantly *lukS/lukF-PV* positive. In the same study, the ST30 clone accounted for 453 isolates (70.4% CA-MRSA and 29.6% HA-MRSA) [4]. These two clones, widely distributed in the community, are introduced on a regular basis in the NICU from the HCWs and people caring for the neonates.

Continuous investigation of MRSA prevalence is useful to uncover reservoirs for on-going MRSA transmission in NICUs and has proved challenging. Well-known nosocomial MRSA clones are being constantly introduced and transmitted through transfers from MHs, parents and HCWs. Effective infection control requires constant vigilance, since the best strategy to avoid neonatal MRSA infections lies in prevention rather than treatment.

Declarations

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Authors contribution: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Anastassios Doudoulakakis, Nikolaos Giormezis, Angeliki Nika, Elisavet Bozavoutoglou, Maria Militsopoulou, Georgios Kalogeras, Evangelia Lebessi. Molecular analysis was performed by Nikolaos Giormezis and Maria Militsopoulou. The first draft of the manuscript was written by Anastassios Doudoulakakis and Garyfallia Syridou and all authors commented on previous versions of the manuscript. Supervision of the study as well review and editing of the study were performed by Iris Spiliopoulou, Maria Tsolia and Evangelia Lebessi. All authors have read and approved the final manuscript.

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