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Major article

Methicillin-resistant *Staphylococcus aureus* nasal colonization in a level III neonatal intensive care unit: Incidence and risk factors

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Key Words:
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Objective: To describe epidemiologic features and identify risk factors for methicillin-resistant *Staphylococcus aureus* (MRSA) acquisition in a level III neonatal intensive care unit (NICU).
Setting: A prospective, cohort study in a university-affiliated NICU with an infection control program including weekly nasal cultures of all neonates.
Methods: Demographic, clinical, and microbiologic data were prospectively collected between June 2009 and June 2013. Molecular characterization of MRSA isolates was done by multilocus variable number tandem repeat fingerprinting, staphylococcal cassette chromosome *mec* typing, and on representative isolates by multilocus sequence typing and *spa* typing.
Results: Of 949 neonates, 217 (22.87%) had a culture growing MRSA, including 117 neonates testing positive at their first sampling. Of these latter infants, 96 (82.05%) were born with the infection and 59 (50.43%) had been transferred from the nursery. Length of stay and colonization pressure were strong independent predictors of MRSA acquisition. Among MRSA isolates, 7 sequence types were identified, with ST22-IVa, *spa* type t223, being the predominant strain.
Conclusions: In an endemic area, early MRSA acquisition and high colonization pressure, likely related to an influx of colonized infants from a well-infant nursery, can support persistence of MRSA in NICUs. Surveillance, molecular tracking of strains, and reinforcement of infection control practices, involving well-infant nurseries in a comprehensive infection control program, could be helpful in containing MRSA transmission.

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major etiologic agent of infection worldwide.[1] In the years 2009-2012 the percentage of invasive isolates resistant to methicillin in the European Union/European Economic Area has shown a decreasing global trend.[2] However, in 2012 the mean MRSA percentage remained as high as 18% and above 25% in 7 countries, mainly in eastern and southern Europe, including Italy.[2]

In adult patients, MRSA infections are becoming less common, whereas infections in neonatal intensive care units (NICUs) seem to be becoming more frequent.[3] Aggressive measures can be necessary to contain the outbreaks, frequently in the form of bundle strategies.[4] However, in highly endemic areas, despite these measures being rigorously enforced, ongoing MRSA transmission and infection have been recorded for many years.[5]

Colonized neonates play a major role as endogenous reservoirs of MRSA in NICU settings.[3] Consequently, active surveillance culture (ASC) programs can be instituted to identify colonized patients and obtain otherwise unavailable information helpful to control MRSA transmission.[3,4]

We recently reported the epidemiologic characteristics and the temporal trend of the endemic MRSA colonization in the level III NICU of the Azienda Ospedaliera Universitaria Policlinico (AOUP) "P. Giaccone," Palermo, Italy, during the period June 2009-June 2012.[6] The purpose of our study was to describe risk factors for

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MRSA acquisition in this NICU during the 4-year period June 2009-June 2013. We analyzed also antimicrobial resistance profiles and molecular genetic characteristics of the MRSA isolates.

METHODS

Study design and setting

We performed a prospective cohort study of MRSA colonization in the level III NICU of the AOUP "P. Giaccone," Palermo, Italy. This NICU annually admits about 250 infants. Because it is associated with the regional reference center for genetic diseases and a neonatal surgery unit, the NICU has a high prevalence of neonates with malformation or complex conditions requiring surgical care (~40%) as well as admissions from other hospitals (~35%). The NICU has 1 intensive care room consisting of 8 cot spaces and 1 intermediate care room with 8 additional cot spaces. The average number of nurses by working shift in the intensive and intermediate care rooms is 2.7 and 2.0 year-round and 2.0 and 1.5 during the summer vacation period. The NICU is open to parents for 2 hours in the morning and 4 hours in the afternoon to allow them to be trained in the general care of their infants. A well-infant nursery is located in the same hospital facility where rooming-in care is routinely performed and early breastfeeding is strongly supported. Ampicillin-sulbactam and gentamicin are the most frequently used antibiotics in the NICU setting.

Inclusion criteria were admission to our NICU between June 16, 2009, and June 15, 2013; hospitalization for at least 48 hours; and collection of at least 1 nasal swab. Colonization was defined as isolation of MRSA from anterior nares without evidence of infection. Infection was defined using the Centers for Disease Control and Prevention National Healthcare Safety Network criteria for postnatally acquired infections.[7]

Demographic characteristics, gestational age, birth weight, inborn or outborn condition, delivery type, Apgar score, and comorbid conditions were recorded at admission. Any prior stay in the nursery was also traced. Clinical and microbiologic data were prospectively collected as qualitative and quantitative data, including the following at-risk exposures: presence of central vascular access devices, endotracheal intubation, nasal continuous positive airway pressure, type of feeding (ie, parenteral nutrition, gavage, breast milk, and formula), surgery, antibacterial drug therapy, length of stay (LOS), and survival status at discharge. Diagnosis related group weight was also included.

The study protocol was approved by the ethics committee of the AOUP "P. Giaccone," Palermo, Italy, and informed consent was sought from the parents or guardians of the neonates.

Infection control strategies

Since June 2009, an ASC program has been in place, including nasal swabs obtained on a weekly basis from all infants staying in the NICU. Measures taken to control MRSA spread in NICU include contact precautions, use of dedicated equipment, cyclic training sessions of health care workers (HCWs), and intensified environmental sanitation. Attention is paid to prevent overcrowding and relative understaffing, minimize hospital LOS, and promote safe use of invasive devices. All infants with MRSA colonization or infection are placed in contact isolation and cohorted, but a dedicated nursing team cannot be guaranteed due to staffing shortages. Routine cleaning policies include postdischarge cot terminal cleaning in the NICU disinfection room, irrespective of the MRSA carriage status of the occupant. Environmental surfaces are not routinely cultured. No neonates are treated with mupirocin for decolonization. Other measures elsewhere described to control MRSA outbreaks, such as chlorhexidine baths or unit closure, have not been carried out.

Active surveillance cultures

Surveillance specimens from the anterior nares of neonates were incubated overnight in brain-heart infusion broth (Oxoid, Basingstoke, UK) and then plated onto mannitol salt agar (Oxoid). Presumptive *S aureus* isolates were identified according to standard methods. MRSA isolates were searched for by colony screening onto oxacillin agar (Mueller-Hinton with oxacillin 6 mg/L) and confirmed by the cefoxitin disk diffusion test and polymerase chain reaction (PCR) for detection of *mec*A.[8]

The first isolate from each patient was submitted to antibiotic susceptibility test and genotyping. Susceptibility testing was routinely performed using the disk diffusion method using *S aureus* ATCC 25923 as the quality control strain. Macrolide-lincosamide-streptogramin B-inducible phenotypes were detected by the D-zone test per European Committee on Antimicrobial Susceptibility Testing guidelines (www.eucast.org/clinical_breakpoints/). Susceptibility testing results were interpreted based on European Committee on Antimicrobial Susceptibility Testing clinical breakpoints.

Molecular typing of MRSA isolates

Staphylococcal cassette chromosome *mec* was typed by a previously described multiplex PCR method.[9] Genotyping of the MRSA isolates was routinely performed by multilocus variable number tandem repeat fingerprinting (MLVF).[10] Banding patterns were analyzed both visually and by using Bionumerics version 5.10 (Applied Maths, Sint-Martens-Latem, Belgium). Moreover, the presence of *lukS/lukF-PV* and *tst1* genes encoding the Panton Valentine leukocidine and the toxic shock syndrome toxin 1, respectively, was tested for by PCR.[11] The ST22-MRSA-IVa isolates were also tested by PCR for the carriage of enterotoxins C and L (ie, *sec, sel*) using previously described primers and conditions.[11]

A subset of representative isolates, including all the different MLVF patterns, was analyzed by multilocus sequence typing (MLST). MLST allelic profiles and sequence types were assigned by submission to the *S aureus* MLST database (www.mlst.net). Additionally, *spa* typing was carried out on representative MRSA isolates.[12] The *spa* type was determined using Ridom StaphType software (http://www.ridom.de/staphtype/).

Statistical analysis

Statistical analysis was performed by using EpiInfo (version 7; Centers for Disease Control and Prevention, Atlanta, Ga) and R software, version 2.13.0 (R Foundation for Statistical Computing, Vienna, Austria). Time intervals at risk for MRSA colonization were defined as the time between admission and date of first nasal swab positive for MRSA colonization in patients testing negative to the first nasal swab, and time between admission and death or discharge for noncolonized infants. Patients testing positive were considered to be MRSA colonized from the date of their first positive sampling until discharge or death. Overcrowding was assessed by calculating the average bed occupancy rate (ie, the percentage of occupied beds per day divided by the number of available beds) during the at-risk stay of each patient. The infant-to-nurse ratio during the at-risk stay of each patient was calculated by using the daily census divided by the number of nurses on duty. Colonization pressure was calculated as the proportion of total patient-days that were MRSA-positive patient-days during the time at risk. All colonized patients contributed to colonization pressure.

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