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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:
Colonization pressure
Active surveillance
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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 inborn 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.

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.

Categorical variables were summarized as frequency (%) and compared by using Pearson χ^2 test, χ^2 test for linear trend, or Fisher exact test, as appropriate. Continuous variables were reported as mean ± SD and compared by using Student *t* tests when normally distributed, otherwise they were presented as median (interquartile range) and analyzed by using the Mann-Whitney-Wilcoxon test.

All variables that were found to be significantly associated ($P < .05$) with acquiring MRSA colonization in the univariate analysis were included in a backward stepwise multivariable logistic regression analysis. The model with the lowest Akaike information criterion was considered to have the best fit and the Hosmer-Lemeshow goodness of fit was used to determine how well the final model fit the data. Adjusted odds ratios and 95% confidence intervals were calculated for the variables retained in the best-fitting models. All reported *P* values were 2-sided and $P < .05$ was considered significant.

RESULTS

General epidemiologic features of nasal colonization with MRSA

During the study period, 999 infants were admitted to the NICU, of whom 949 fulfilled the criteria for inclusion in the study. There were no significant differences in the number of enrolled infants among the 4 years of study.

As shown in Table 1, of the 949 patients, 354 (37.30%) were outborn and 173 (18.23%) had malformation. Moreover, 225 (23.71%) neonates had been admitted to the nursery before being transferred to the NICU. Median LOS in the NICU was 11 days (range, 2-178 days). The main characteristics of the 949 infants at admission are summarized in Table 1.

During the 4-year period, 2,549 nasal swabs were cultured (mean, 2.69 per patient). The first nasal swab was obtained on average 3.91 days after admission (4 [1-6] days). Overall 217 neonates (22.87%) had a nasal surveillance culture growing MRSA. Of these, 117 (53.92%) tested positive at the time of their first sampling, whereas the remaining 100 (46.08%) acquired MRSA later during their NICU stay. The interval of time between admission and collection of the first nasal swab from the NICU infants did not differ significantly between the 2 subgroups (4 [2-6] days for those with the first swab positive vs 3 [1.5-6.5] days for those with the first swab negative; $P = .10$).

Table 2 shows the differential characteristics of the 2 subgroups of patients—MRSA colonized and noncolonized—at their first nasal sampling, at the NICU admission. A significantly higher proportion of infants being inborn and coming from the nursery was found among the patients testing positive at their first nasal swab compared with those who did not.

Incidence and risk factors for MRSA acquisition

The mean quarterly incidence density was 6.84 cases per 1,000 patient-days (95% confidence interval, 5.62-8.31). The median (interquartile range) quarterly colonization pressure was 15.34% (5.44%-27.44%). Figure 1 shows that during the study period an overall decreasing trend was evident with colonization incidence density approximately halving; that is, from 14.86 to 7.86 cases per 1,000 patient-days. This trend was interrupted by a transient increase during the fifth quarter and by a subsequent major peak of incidence/colonization pressure, involving the eighth to 10th quarters. As previously reported, the import in the NICU of a MRSA strain ST1-IVa followed by a period of substantial overcrowding were the probable driving factors.[6] The declining trend came again to a halt in the 14th quarter concurrently with the emergence in the NICU of a strain of *Klebsiella pneumoniae* carbapenemase-producing *K pneumoniae* (KPC-Kp).[13] Indeed, between September 18, 2012, and November 14, 2012, KPC-Kp was recovered from 10 neonates. The sudden requirement for a prioritized cohorting of the patients colonized with KPC-Kp placed a major burden on the nursing staff and made it necessary to convert the separate intermediate care room into a KPC-Kp cohort room, while cohorting of patients positive for MRSA infection was forcedly restricted.[13]

A seasonal variation was evident for MRSA colonization with incidence density peaking in the summer and autumn quarters (June-November).

Table 1
General characteristics of patients at admission to the neonatal intensive care unit (June 2009-June 2013, Palermo, Italy)

Variable	Result
Total	949 (100)
Gender	
Male	547 (57.64)
Age at admission, h	
<24	728 (76.61)
24-48	114 (12.01)
>48	105 (11.06)
Gestational age, wk	
<30	43 (4.53)
30-36	312 (32.88)
>36	588 (61.96)
Patient transferred from nursery	225 (23.71)
Birth weight, g	
≤1,000	28 (2.95)
1,001-1,500	53 (5.58)
1,501-2,000	112 (11.80)
2,001-2,500	171 (18.02)
>2,500	580 (61.12)
Inborn	595 (62.70)
Twin birth	123 (12.96)
Cesarean delivery	623 (65.65)
Apgar score at 5 min < 8	97 (10.22)
Malformation	173 (18.23)

NOTE. Values are presented as n (%).

Table 2
Characteristics of patients in the neonatal intensive care unit stratified by methicillin-resistant *Staphylococcus aureus* (MRSA) colonization status at their first nasal swab (June 2009-June 2013, Palermo, Italy)

	First nasal swab		
Variable	MRSA negative	MRSA positive	*P* value
Total	832 (87.67)	117 (12.33)	
Gender			
Male	484 (58.17)	63 (53.85)	.37
Age at admission, h			
<24	628 (75.48)	100 (85.47)	.059
24-48	106 (12.74)	8 (6.84)	
>48	96 (11.54)	9 (7.69)	
Gestational age, wk			
<30	42 (5.05)	1 (0.85)	.008
30-36	283 (34.01)	29 (24.79)	
>36	666 (80.05)	86 (73.50)	
Patient transferred from nursery	166 (19.95)	59 (50.43)	<.001
Birth weight, g			
≤1,000	28 (3.37)	0 (0)	.008
1,001-1,500	51 (6.13)	2 (1.71)	
1,501-2,000	99 (11.90)	13 (11.11)	
2,001-2,500	150 (18.03)	21 (17.95)	
>2,500	500 (60.10)	80 (68.38)	
Inborn	499 (59.98)	96 (82.05)	<.001
Twin birth	111 (13.34)	12 (10.26)	.34
Cesarean delivery	549 (65.99)	74 (63.25)	.53
APGAR score at 5 min < 8	92 (11.06)	5 (4.27)	.02
Malformation	158 (18.99)	15 (12.82)	.09

NOTE. Values are presented as n (%).

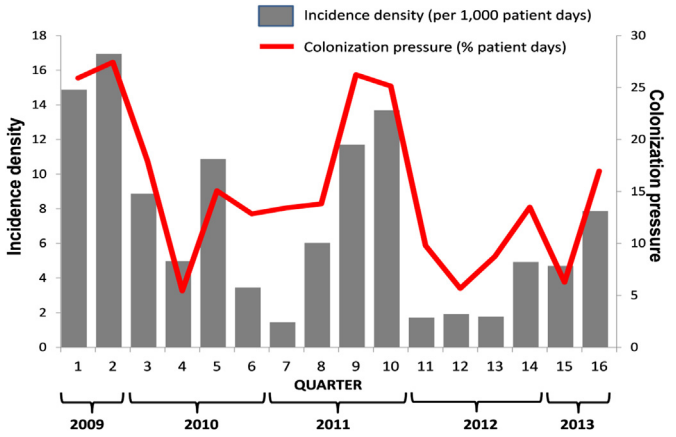

Fig 1. Distribution of incidence density of methicillin-resistant *Staphylococcus aureus* (MRSA) acquisition and MRSA colonization pressure rates by quarter during the study period.

MRSA acquisition analyses included only the 832 participants whose first nasal swab was negative (Table 3). No statistically significant difference was found between the infants who acquired MRSA and those who did not when comparing the proportion of patients who were inborn or transferred to the NICU from the nursery. The median LOS as well as the mean of percent colonization pressure were both significantly higher for colonized infants. Conversely, the difference between these 2 subgroups was not statistically significant for daily occupation rate and infant-to-nurse ratio.

In accordance with the results of the multiple logistic regression analysis (Table 4), MRSA acquisition was negatively associated with male gender, an increasing birth weight, and systemic antibacterial therapy. Conversely, LOS and colonization pressure were confirmed to be strong independent risk factors for MRSA acquisition: indeed, for every day and percent unit of increment, the odds ratio of becoming colonized with MRSA increased by 4% and 5%, respectively.

Twenty-eight of 100 infants (28.0%) who acquired MRSA during their NICU stay developed late-onset sepsis compared with 59 out of 732 (8.06%) who did not ($P < .001$). A total of 23 infants (2.81%) died during their NICU stay, but in-hospital mortality rate was not significantly different between the 2 subgroups of patients (2.17% vs 2.89%; $P = .69$).

Characteristics of the MRSA isolates

Two hundred seventeen isolates from 216 patients were available for typing. One infant only was shown to simultaneously carry 2 different strains (ST22-IVa and ST1-IVa). Overall, 7 sequence types (STs) were identified (ST1, ST7, ST8, ST20, ST22, ST45, and ST97) with ST22-IVa being the predominant (190 out of 217; 87.56%). SCC*mec* type IVa was identified in all MRSA isolates. ST22-IVa isolates were further subdivided by MLVF into 9 different subtypes, of which only the subtype ST22-A was detected through the entire period of the study. The second most common ST was ST1, which was detected in 20 isolates. ST1 was responsible for 2 epidemic spreads, of which the first was initiated by the import of an outborn infected patient, involved 15 infants, and made necessary a strengthening of the infection control practices to interrupt transmission.[14] They were assigned with type t127 by *spa* typing.

The MRSA isolates were fully susceptible or resistant to a limited number of non-β-lactam antibiotics. Only the ST1-IVa isolates showed an inducible clindamycin-resistant phenotype. No isolate was found to carry the Panton Valentine leukocidine gene sequence. The *tst1*, *sec,* and *sel* gene sequences were detected in all ST22-MRSA-IVa isolates. These isolates were assigned to type t223 by *spa* typing.

Table 3
Factors associated with acquisition of methicillin-resistant *Staphylococcus aureus* (MRSA) in patients testing negative at the first nasal swab, June 2009-June 2013, Palermo, Italy

Variable	Infants who acquired MRSA No. 100 (12.02%)	Infants who did not acquire MRSA No. 732 (87.98%)	*P* value
At hospital admission			
Male gender	42 (42.0)	442 (60.38)	<.001
Twin birth	22 (22.0)	89 (12.16)	.005
Malformation	30 (30.0)	128 (17.49)	.003
Inborn	65 (65.0)	434 (59.29)	.27
Birth through cesarean section	80 (80.0)	469 (64.07)	.003
Admission to neonatal intensive care unit < 24 h after birth	83 (83.0)	545 (74.45)	.07
Apgar score at 5 min <8	18 (18.0)	74 (10.11)	.03
Gestational age, wk	35.5 (32-38)	37 (35-39)	<.001
Birth weight, g	2,170 (1,420-2,770)	2,775 (2,190-3,265)	<.001
Patient transferred from nursery	16 (16.0)	150 (20.49)	.29
During hospital stay			
Central venous access device			
No	49 (49.0)	472 (64.5)	<.001
Yes, 1-14 d	23 (23.0)	171 (23.4)	
Yes, >14 d	28 (28.0)	87 (11.9)	
Endotracheal tube			
No	61 (61.0)	582 (79.5)	<.001
Yes, 1-3 d	16 (16.0)	51 (7.0)	
Yes, >3 d	23 (23.0)	98 (13.4)	
Nasogastric tube			
No	38 (38.0)	462 (63.1)	<.001
Yes, 1-14 d	18 (18.0)	159 (21.7)	
Yes, > 14 d	43 (43.0)	107 (14.6)	
Nasal continuous positive airway pressure			
No	58 (58.0)	599 (81.8)	<.001
Yes, 1-3 d	14 (14.0)	71 (9.7)	
Yes, > 3 d	28 (28.0)	61 (8.3)	
Parenteral nutrition	72 (72.0)	472 (64.48)	.14
Surgical procedure	14 (14.0)	69 (9.43)	.13
Systemic antibacterial therapy			
No	36 (36.0)	297 (40.57)	.001
Yes, 1-7 d	15 (15.0)	213 (29.10)	
Yes, > 7 d	49 (49.0)	220 (30.05)	
Formula feeding	98 (98.0)	683 (93.31)	.13
Breast milk feeding	57 (57.0)	383 (52.32)	.95
Diagnosis-related group weight	1.58 (0.70-5.6)	0.76 (0.72-3.25)	.0065
Length of stay, d	15.0 (9-26)	10 (7-19)	<.001
Daily bed occupancy rate %	81.2 (68.7-87.5)	75 (62.5-81.2)	.61
Infant-to-nurse ratio	3.4 (2.6-3.8)	3.1 (2.2-3.7)	.63
Colonization pressure %	18 (9.5-26)	12 (8-19)	<.001

NOTE. Data are presented as n (%) or median (interquartile range).

DISCUSSION

We investigated the epidemiologic pattern of MRSA colonization in a level III university-affiliated NICU where an endemic presence of a strain characterized as *tst*1 positive, UK-EMRSA-15/"Middle Eastern Variant" had been previously documented.[6]

High incidence density and colonization pressure characterized our NICU setting through the 4-year study period. After the

Table 4
Best fitting logistic regression model, by Akaike information criterion, for predicting the risk of colonization with methicillin-resistant *Staphylococcus aureus* (Hosmer-Lemeshow goodness-of-fit test $P = .21$)

Variable	Adjusted odds ratio (95% confidence interval)	*P* value
Gender, male vs female	0.60 (0.37-0.97)	.038
Birth weight, per 100 g increase	0.96 (0.93-0.99)	.047
Malformation, yes vs no	1.77 (0.98-3.19)	.062
Systemic antibacterial therapy, per day increase	0.97 (0.95-0.99)	.026
Length of stay, per day increase	1.04 (1.02-1.05)	<.001
Colonization pressure %, per unit increase	1.05 (1.02-1.07)	<.001

reinforcement of the infection control procedures, an overall downward trend was registered.

Screening at admission was not performed. However, a proportion of colonized infants as high as 53.92% was shown to be positive at their first nasal swab, the majority being inborn. This finding is unexpected based on the available literature. A recent meta-analysis shows, indeed, a significantly higher colonization rate among outborn infants, a plausible consequence of their older age and more prolonged exposure to the health care setting.[3] In addition, about 50% of infants testing positive at their first nasal swab had been transferred from the nursery. MRSA transmission is already known to be highly efficient in nurseries due to the routine care practices and a lower awareness by HCWs of general infection control measures.[15] Unfortunately, our ASC program was not extended to the nursery, preventing us from drawing definitive conclusions.

In accordance with previous observations, a seasonality of MRSA colonization was apparent in our setting. This phenomenon has been reported in a significant proportion of studies about MRSA infection, particularly as an association of warmer seasons with skin and soft-tissue infections.[16] Higher incidences in the summer and autumn have been specifically observed for community-associated MRSA.[16] Comparatively few investigations have focused on nasal colonization, with nondefinitive conclusions.[16] MRSA seasonality deserves to be thoroughly studied. Actually, seasonality could be the epiphenomenon of many concurrent, specific variables, such as overcrowding and understaffing (eg, staffing shortage during summer holidays) along with pressure by MRSA carriage in the community in highly endemic areas, such as the southern European countries.[16]

In our setting colonization pressure was shown to be a powerful risk factor, odds of acquiring MRSA being 5% higher per unit increase. Conversely, overcrowding and understaffing did not play a key role in promoting MRSA acquisition. Previous reports have repeatedly emphasized the role of a high colonization pressure, which has been shown to be able to supersede the effects of other transmission variables, including infection control measures.[17,18] Moreover, it is widely acknowledged that once colonization pressure is high, it becomes the major variable affecting MRSA acquisition.[17,18]

A growing role of community-associated MRSA strains is reported in NICUs.[6,19,20] However, a meaningful distinction between health-care associated MRSA and community-acquired MRSA strains is increasingly challenging and MRSA strains previously identified as belonging to either group are reported to circulate in both community and health care settings.[14,21] Moreover, highly endemic community settings are likely to promote spilling of MRSA into the NICUs via HCWs or parents.

The endemic strain detected in our NICU was the *tst*1 positive, UK-EMRSA-15/"Middle Eastern Variant," which was first described by Biber et al[22] as genetically related to the epidemic EMRSA-15 clone. Like in Gaza, in our geographic area a question arises about the origin of this clone; that is, a health care-associated MRSA escaping toward the community or, alternatively, an ST22-MSSA that has evolved into a community-acquired MRSA clone. The detection in healthy children in the community of this MRSA strain and of an ST22-MSSA-*spa*-t223 strain (unpublished data) appears to be more supportive of this latter hypothesis.[23] Other strains entered the NICU in a sporadic or epidemic way during the study period, all with characteristics of community-acquired MRSA strains. In particular, ST1-MRSA-IVa isolate *spa* type t127 was detected as responsible for 2 different events.[14] This MRSA lineage is considered to have a high zoonotic potential and has been recently reported in Italy, mainly from fattening pigs.[24]

As found by other investigators, in NICUs with a high endemicity and multiple circulating MRSA strains, the objective to definitively eradicate MRSA from the NICU can be unrealistic.[5,25]

With a 4-year period of prospective epidemiologic and laboratory surveillance and molecular typing of MRSA isolates, our study provides significant data about risk factors for acquisition and evolution of MRSA clones in a level III NICU. Molecular typing is critical to support effective surveillance and control strategies. MLVF, in particular, has been shown to be a very useful, high-throughput, and cheap tool to rapidly discriminate the MRSA isolates and timely detect endemic, epidemic, and unrelated strains.

Nonetheless, our study has many limitations. Screening at admission to our NICU was not made, which prevented us from more accurately detecting time and place of MRSA acquisition and defining the role of the well-baby nursery and of outborn admissions. Moreover, in accordance with the objective of our study, infants staying in the nursery did not undergo nasal sampling. Neither HCWs nor family members were screened for MRSA, which could have resulted in unidentified entry or transmission routes. However, the optimal approach of active surveillance testing to detect MRSA carriage screening among HCWs has not been definitively agreed upon and active surveillance is only recommended for implementation in response to clusters of colonization or infection cases with a suspected epidemiologic link to an HCW.[4,26] Likewise, the role of parents in importing MRSA into NICU settings requires further study. Moreover, the presence of possible environmental reservoirs has not been explored due to conflicting evidence about the contribution of persistent environmental MRSA contamination in health care settings.[26] Extranasal sites were not considered in the ASC program because nasal cultures only are widely agreed to be sufficiently sensitive.[3,4] Moreover, molecular testing by PCR was not adopted due to the inconclusive evidence about its cost-effectiveness when measured by the MRSA acquisition rate[26] and the need to maintain the standard culture-based approach for the purpose of typing the MRSA isolates. A correlation between colonization and sepsis by MRSA was not attempted because of the low yield of blood cultures in neonates.[27] Finally, because this was an observational study conducted in a single NICU in an university-affiliated hospital, the results may not be generalizable.

CONCLUSIONS

Our study results suggests that in a hyperendemic area early MRSA acquisition and high colonization pressure, likely related to a constant influx of colonized infants from a well-infant nursery, can support persistence of MRSA in NICU settings and substantially interfere with infection control strategies. Our findings highlight also some further concerning features of epidemiology of MRSA in NICU settings, such as the recurrent emergence of sporadic or clustered MRSA strains occurring amidst a background of an endemic MRSA strain. Careful vigilance with surveillance, molecular tracking of strains, and the reinforcement of infection control

practices involving well-infant nurseries in a comprehensive infection control program could be helpful in containing MRSA.

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