Surveillance on methicillin sensitive Staphylococcus aureus colonization and infection in a neonatal intensive care unit

Title

Surveillance on methicillin sensitive *Staphylococcus aureus* colonization and infection in a neonatal intensive care unit.

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Keywords: Colonization, Surveillance, Neonatal Intensive Care Unit, Infection, Neonate, *Staphylococcus aureus*

Summary

Background:

Methicillin sensitive *Staphylococcus aureus* (MSSA) infection is a significant health concern in neonatal intensive care units (NICUs). Bacterial colonization increases the risk of subsequent infection, leading to morbidity and mortality.

Aim:

To report the findings of a retrospective cohort study on the surveillance of MSSA colonization and infection in NICU patients.

Methods:

The weekly microbial surveillance results for MSSA colonization in the throat, nose, anus, and groin, as well as invasive and non-invasive MSSA infections, were analyzed from November 2020 to June 2022. The MSSA infection and colonization risk were compared after adjustment for confounders by stepwise logistic regression analysis.

Findings:

Three hundred eighty-three neonates were screened; 42.8% (*N*=164) were MSSA colonized. Significant risk factors for MSSA colonization were length of stay, vaginal delivery and extreme low gestational age <28 weeks [ELGAN] (all $P < 0.05$). The surveillance detected 38 (9.9%) mild MSSA infections and 11 (2.9%) invasive MSSA infections. Neonatal colonization with MSSA is a major risk factor for MSSA infection overall (29.3% in colonized/infected vs. 70.7% colonized/not-infected and 0.5% in not-colonized/infected vs. 99.5% in not-colonized/not-infected infants) and invasive MSSA infections (6.1% in colonized/infected vs. 93.9% in colonized/not-infected and 0.5% in non-colonized/infected vs. 99.5% not-colonized/not-infected infants). Also, extreme low birth weight (<1000g), ELGAN and invasive ventilation were significant risk factor for MSSA infections (all, $P < 0.05$).

Conclusions:

The link between postnatal MSSA colonization and subsequent MSSA infection offers possibilities for prevention. Additional research is needed to explore the association between vaginal birth and the pathogenesis of neonatal MSSA colonization.

Keywords: Colonization, Surveillance, Neonatal intensive care unit, Infection, Neonate, Staphylococcus aureus

Introduction

Staphylococcus aureus poses significant health risks in NICUs, particularly for extremely low gestational age neonates < 28 weeks (ELGANs) with underdeveloped immune systems and frequent exposure to healthcare settings [1-3]. Colonization with *S. aureus* increases the likelihood of subsequent infections, leading to significant morbidity and mortality in this vulnerable population [4]. *S. aureus* is currently the second most common pathogen causing bloodstream infections and the leading cause of ventilator-associated pneumonia in NICUs [5]. While many NICUs, including ours, conduct methicillin-resistant *S. aureus* (MRSA) colonization screenings and implement precautions for MRSA carriers, such as cohort isolation and single rooms, there is limited epidemiological data on methicillin-sensitive *S. aureus* (MSSA) colonization and infection in NICUs [6]. Some studies have focused on eradicating MSSA colonization in very low birth weight infants to prevent MSSA infections [7, 8].

This study aimed to improve our understanding of the epidemiology and clinical impact of MSSA colonization and infection. The cohort consisted of neonates admitted to a NICU in Belgium during a specific period. Data were collected from electronic medical records and the hospital laboratory database to determine the prevalence of *S. aureus* colonization, the incidence of *S. aureus* infections, and associated risk factors. The study's detailed analysis provides valuable insights into the epidemiology, risk factors, and clinical outcomes related to *S. aureus* colonization and infection in NICU settings. The results of the study have important implications for the management of neonates in NICUs, including the need for effective infection control measures and the judicious use of decolonization strategies in order to prevent infections in NICU patients.

Methods

Setting

This retrospective cohort study was performed in NICU patients admitted from November 2020 and discharged before June 2022 (20 months) at the Antwerp University Hospital (UZA) in Belgium. The UZA NICU is a level III unit with 32 beds (28 high care and 4 low care beds) and with approximately 450 admissions a year including low care admissions. As a reference center there is a high proportion of intra uterine transfer (35% approximately) and a high rate of extreme low gestational age neonatal admissions (12% approximately) and very low birth weight infants (<1500 gram): 34%. Standard hand hygiene practices for healthcare workers, parents, visitors, and legal guardians consists of using hand alcohol before and after each patient contact and in the immediate patient environment. Except for of a facemask due to standard universal COVID isolation precaution, gowns and gloves are not mandatory unless the patient is under contact isolation precautions. An MRSA screening was implemented in the UZA NICU since 1999 and was extended to MSSA since 2019. All neonates were screened weekly on Tuesday, and not particularly at the day of admission, until discharge with one swab at the throat, anterior nares, anus and groin (TNAG). For this procedure flocked nylon swabs were transported to the microbiology laboratory in Amies medium (eSwab, Copan). Swabs were plated on a chromogenic SA*Select* agar and MRSA*Select* II agar (BioRad) for MSSA and MRSA detection respectively. Colony identification was performed with MALDI-TOF mass spectrometry (MALDI Biotyper, Bruker) and antibiotic susceptibility testing via disk diffusion using EUCAST clinical breakpoints [9].

Infants with clinical symptoms of infection were sampled according to the clinical decision.

Study population

Inclusion criteria were admission within 72h after birth (to exclude readmission), a hospital stay of at least 7 days and at least one MSSA surveillance swab. Patients colonized with MRSA were excluded.

Infants were categorized as MSSA colonized definitive when at least one TNAG culture tested positive. Infections with MSSA were categorized as invasive (MSSA isolated from blood and/or lower respiratory tract) or mild (MSSA isolated from conjunctiva or skin/wound). Bloodstream infection and ventilation associated pneumonia (VAP) were defined according to the definitions of NeoKISS surveillance system [10].

Data collection

Patient's demographic characteristics, gestational age, birth weight, sex, inborn or outborn origin, delivery mode and patient's treatment conditions, presence of central line and central line days, non-invasive ventilation days, endotracheal intubation, invasive ventilation days, length of hospital stay, were retrieved from the electronic patient information system Metavision Suite 6.0 (Tel Aviv, Israel) or Millenium (Oracle Cerner, Kansas City, US). The MSSA culture data were retrieved from the laboratory information system Molis (Compugroup Medical, Oostend, Belgium).

Statistical analysis

The characteristics of the NICU patients with and without MSSA colonization and infection were compared by calculating the median (interquartile range, IQR) and frequencies. The significance of difference was tested by Kruskal-Wallis (K-W) test, or chi-square test, respectively. All tests of significance were 2-tailed and *P*-values < 0.05 were considered statistically significant. A forward stepwise multivariable nominal logistic regression was performed to determine the factors independently associated with development of MSSA colonization and MSSA infection. Only factors with a *P*-value < 0.25 in the univariate analysis were entered in the multivariate analysis. For the statistical analysis JMPpro 17.1 (SAS, NC, USA) was used. No sample size or power calculation was performed.

Ethics

The study (EDGE n° 002937) was approved by the review board of the University Hospital of Antwerp.

Results

During the study period 383 neonates fulfilled the inclusion criteria. One neonate was excluded because of MRSA colonization. Their characteristics are summarized in Table I. Of the 383 screened infants 164 (42.8%) became colonized with MSSA during their hospital stay: 48 (29.3%) MSSA colonized neonates developed MSSA infection. While in the non-MSSA colonized cohort only 1 neonate developed an invasive MSSA infection (Odds Ratio [OR] 90.2, *P* < 0.001). Of the 49 MSSA infected patients 11 (22.4%) had an invasive infection [6.1% of the MSSA colonized NICU patients]. Of these 7 were ventilator associated pneumonia and 4 bloodstream infections. However, mild MSSA infections such as conjunctivitis [n=19], skin and wound infections [n=19] were more frequently encountered [n=38, 77.6%]. The median time delay between MSSA colonization and infection was 8 days (IQR 4-19 days). The median hospital day of MSSA colonization was 11 days (IQR 7-17 days), while median hospital day of MSSA infection was 21 days (IQR 10.75-31.2) with no differences between invasive and mild MSSA infections (median 22 days vs 21 days; K-W X^2 0.83, *P* = 0.36). The case fatality rate of invasive MSSA infection was 18% (2/11), while 0% for mild MSSA infections.

Factors associated with MSSA colonization

The characteristics of MSSA colonized and not-colonized infants are summarized in Table II. Compared to not colonized MSSA patients, NICU patients with MSSA colonization were more in the ELGAN category and as a consequence had more CVC use, shorter hospital stay without infectious events until discharge. There were significantly more MSSA colonized infants observed in the vaginal delivered infants. This trend was more pronounced in ELGANs (MSSA colonization rate in vaginal delivery 89% vs. 65% after cesarean section, compared to non-ELGANs MSSA colonization rate in vaginal delivery 42% vs. 35% after cesarean section). This positive interaction between mode of delivery and gestational age on MSSA colonization rate was significant ($X^2 = 4.92$, $P < 0.05$) after multivariate analysis (Figure1).

During the NICU stay respiratory support such as non-invasive ventilation and intubation were not significantly different between the two groups. After stepwise multivariable logistic regression analysis, risk factors associated with MSSA colonization during the NICU stay were duration of disease-free hospitalization, vaginal delivery, birth weight below 1000 gram (ELBWI) and ELGAN while ELGAN being the strongest risk factor (Table II). Neonates born vaginally became colonized with MSSA earlier in life compared to those born after cesarean section (median 8 days [IQR: 5 – 17 days] vs. median 11 days [IQR 8 - 17 days], K-W chi-square 6.51, $P = 0.01$). The proportion of infants colonized within the first week of admission was significant different (30/101 [29.7%] vs. 21/134 [15.7%], X^2 6.67, $P < 0.05$) between vaginally born vs. cesarean section born infants. The postnatal MSSA colonization dynamics were different among the birth weight categories with a more initial steady increase during the first weeks of hospitalization in the smaller (ELBWI) infants. However, this interaction was borderline not statistically significant, (β -0.09 [95%CI; -0.19 - +0.007], $P= 0.07$). All except one infected infant was MSSA colonized before time of infection and 26.9% (44/164) of the MSSA colonized infants became MSSA-negative before discharge from NICU.

Factors associated with MSSA infection overall

Table III presents a comparison of characteristics between neonates infected with MSSA and those not infected with MSSA. Of the 383 neonates, 49 (12.8%) were infected with MSSA. Neonates infected with MSSA were more likely to be girls, inborns, to be ELBWI , to be an ELGAN, to have a CVC and to be colonized with MSSA. However, after adjustment for confounders by stepwise logistic regression analysis four factors were found to be independently associated with MSSA infection in the NICU, in order of importance: MSSA colonization, this variable had the highest OR (27.49), extreme low birth weight, intubation and duration of hospitalization (Table III). Indeed, in the absence of MSSA colonization the risk of MSSA infections in these NICU patients is extremely low (0,5%) in comparison to those with MSSA colonization (infection rate overall 29.3%).

Factors associated with invasive MSSA infection

Of the 383 neonates, 11 (2.9 %) developed an invasive MSSA infection. Factors specifically associated with invasive MSSA infections are presented in Table IV. Some demographic characteristics at admission which were associated with MSSA invasive infection in the bivariate analysis such as birth weight, treatment associated risk factors e.g. the use of central lines were no longer risk factors after adjustment for confounders such as duration of hospitalization. The adjusted Odds Ratio after multivariable logistic regression of all factors associated with invasive MSSA infection are presented in Table IV. Those were, in ranking order of importance, invasive ventilation (OR, 135; $P < 0.001$), MSSA colonization (OR, 20.78; $P < 0.05$), ELGAN (OR, 15.59; $P < 0.05$), length of hospital stay (OR, 0.95; $P < 0.05$). Neonates with MSSA colonization have an invasive MSSA infection rate of 6.1% in comparison of < 0.5% in their non-colonized counterparts.

The results regarding the performance of MSSA surveillance cultures in predicting MSSA infection among NICU patients, as well as the impact of timing and gestational age, are presented in Table V. Univariate analysis demonstrated that for both invasive and overall MSSA infections, the sensitivity and specificity notably decrease after the initial week of hospitalization. However, the negative predictive value remains consistently high throughout the entire duration of the hospital stay. The positive predictive value (PPV) of an MSSA surveillance culture is consistently highest following the first week of hospital admission. This trend aligns with the prevalence of infectious complications, particularly among neonates with a high likelihood of infections (such as ELGAN patients, with a PPV of 56% for MSSA infection overall and PPV of 20% for invasive MSSA infections).

Discussion

The study focused on identifying the risk factors and dynamics of MSSA colonization and infection among neonates in the NICU. The NICU under investigation is a level-III unit explaining the high percentage of very low birth weight infants and ELGANs, who are at an increased risk of MSSA colonization due to their prolonged hospitalization.

The overall MSSA colonization rate among neonates in the NICU was found to be 43%, which is significantly higher than previously reported rates (17-22%). This disparity can be attributed to variations in sampling methods and sites. Unlike earlier studies that focused on sampling a single site, such as the anterior nares, or limited sites such as the nasal nares and perianal region, this study highlights the importance of screening multiple sites, including the nasopharynx and skin, to enhance the sensitivity of active surveillance cultures [1, 4, 11]. We explored both the timing and the frequency of the surveillance cultures in more detail.

These data suggest that MSSA screening during the first week of admission in a NICU is highly sensitive and moderately specific to predict both invasive and non-invasive MSSA infection in neonates. However, we do not know whether we can improve the specificity by culturing selective body sites (e.g. upper airway only) as was studied by others [12]. Although, difficult to compare with others due to differences in design, methods and study population, our findings are not in accordance with a previous study in which culture results more remote from the time of the onset of pneumonia were less accurate [13]. Based on this data, we recommend initiating MSSA surveillance promptly after birth. Once the patient tests positive for MSSA, it is advisable to discontinue the weekly surveillance cultures. Whether using swabs only from the nares and throat, without including the anal and skin sites, would enhance the specificity of MSSA screening for MSSA infections (mainly respiratory), while maintaining its sensitivity, requires additional clarification [14].

Vaginal delivery was identified as a significant risk factor for MSSA colonization. Indeed, neonates born vaginally not only exhibited a higher risk, they were also more frequently colonized earlier in life. We hypothesize that the transmission of MSSA from mother to child might occurs during birth, with the mother's genital tract being a potential source of MSSA. Although asymptomatic colonization in pregnant women has been reported, there is a lack of research on the vertical transmission risk from the maternal genital tract [15, 16]. Future studies should consider collecting maternal vaginal cultures for MSSA and gather data on factors such as preterm premature rupture of membranes (pPROM) and maternal antimicrobial therapy, which may increase the risk of maternal vaginal dysbiosis [17].

The dynamics of MSSA colonization were found to be different in the ELGAN population. Vaginal birth had a more pronounced effect on the risk of MSSA colonization in ELGANs. It is speculated that the higher incidence of chorioamnionitis and pPROM in ELGANs, along with increased antibiotic utilization during pregnancy, could lead to dysbiosis of the vaginal microbiome, favoring the colonization of

pathogenic flora such as *S. aureus* and Group B Streptococcus [18]. Additionally, the mode of delivery played a role, as ELGANs were more frequently delivered by cesarean section, potentially protecting them from early MSSA colonization by bypassing the maternal vaginal tract. Also others demonstrated perinatal maternal MRSA and MSSA vaginal colonization and vaginal birth as a potential source of neonatal colonization [19-21].

Although the risk of MSSA colonization was high, the incidence of invasive MSSA infections among neonates in the study cohort was relatively low (2.9%) and moderate for mild infections (10.4%). However, neonates colonized with MSSA during admission were more likely to develop MSSA infections of any type, irrespective of their gestational age, birth weight, length of stay, or invasive procedures. This finding aligns with previous studies conducted in neonatal populations [1, 4, 22].

During the initial weeks of NICU admission, neonates are particularly vulnerable to infectious complications due to exposure to invasive procedures like central vascular lines, endotracheal tubes, and surgical interventions. The median onset of nosocomial sepsis in NICU patients is typically 16 days [23]. This explains why the hospital stay prior to infection is shorter in patients with MSSA infections.

The study identified intubation and ventilation as risk factors for invasive MSSA infection, while non-invasive ventilation strategies, such as continuous positive airway pressure (CPAP) and high-flow nasal cannula, may offer protection against *S. aureus* infections. This finding is consistent with other epidemiological studies showing that CPAP-associated pneumonia is a rare complication [10, 24]. It was observed that nasopharyngeal colonization during intubation may directly introduce *S. aureus* into the lower airway. However, oropharyngeal colonization is also common in neonates, which may explain why oral intubation does not decrease the risk of pneumonia as observed in adults [25]. Notably, the duration of ventilation did not appear to increase the risk of infection, indicating that lower airway infection is more likely acquired during the intubation process. Further research is necessary to explore the efficacy of decolonizing the upper airway for *S. aureus* before or at the time of intubation to reduce the incidence of ventilator-associated pneumonia, particularly in NICU patients [26-28]. Finally, we observed that in the absence of MSSA colonization the risk of both mild and invasive MSSA infections in these NICU patients is extremely low (<0.5%) in comparison to those with MSSA colonization (overall infection rate 29% and invasive infection rate 6.1%). Therefore, at the onset of a Gram-positive infection, it appears prudent to perform antibiotic stewardship by implementing an empirical antimicrobial switch from flucloxacillin to vancomycin in the absence of MSSA colonization. This switch aims to provide coverage against methicillin-resistant coagulase-negative staphylococci and/or enterococci, pending the availability of definitive culture results. This approach is particularly relevant for NICUs that do not initiate vancomycin empirically for suspected nosocomial infections [29].

We propose that the targeted prevention of MSSA colonization, such as early nasopharynx and skin disinfection in vaginally born ELGANs, along with MSSA decolonization approaches like upper airway decolonization in ELGANs prior to intubation, could prove to be effective strategies in preventing these infections. However, this requires further exploration and efficacy trials before being applied in general practice. This has been investigated in previous studies with various results in the context of very low birth weight infants in a NICU and in ventilated PICU patients [30, 31].

This study has multiple strengths. Neonates from all birth weight categories participated in the study, which makes the study population representative for other NICUs. The authors had access to all detailed information of cultures from the hospital laboratory information system. To our knowledge, this study is the first to explore the relationship between the dynamics of MSSA colonization and MSSA infections in neonates. Moreover, we study the effect of colonization not only on invasive but also on

non-invasive neonatal infections. However, this study had several limitations. First, the data needs validation in a multicenter study. Second, due to retrospective observational nature of the study, causation and association is difficult to differentiate, especially in the absence of molecular microbial analysis.

In conclusion, this surveillance study highlights the high overall colonization rate of MSSA among neonates in the NICU and identifies that ELGANs, ELBWIs and especially vaginal delivery are potential risk factors. Neonates who become colonized with MSSA early in their hospitalization have the highest risk of developing invasive infections, indicating the importance of implementing infection prevention measures during this critical period. Further research is needed to investigate the transmission of MSSA during the perinatal period. By gaining a better understanding of the risk factors and dynamics of MSSA colonization and infection, healthcare providers can develop targeted strategies to prevent and manage these infections, ultimately improving the outcomes for neonates in the NICU.

Authors' Contributions

All authors have read the manuscript, provided a critical review and approved the final version. LM, EH, AE, VM designed the study, analyzed and interpreted the data. Dr. VM, LM, EH, AE were involved in data collection and data cleaning from the electronic health record and microbiology laboratory database. LM, KVD drafted the manuscript.

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Conflict of interest statement

None

Funding statement

None

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Figure 1.

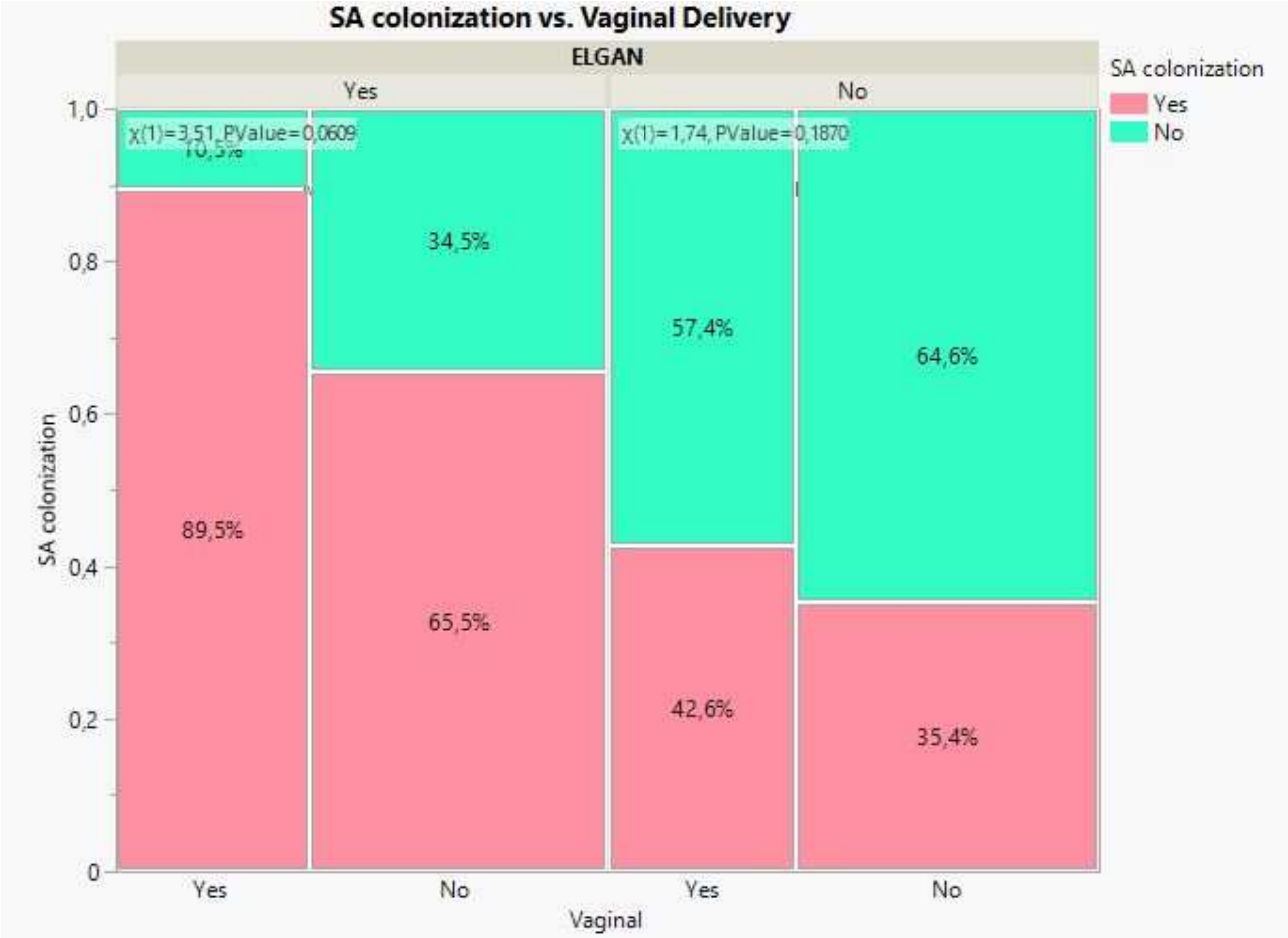

Figure Legends

Figure 1.

Figure title:

Association between vaginal birth and methicillin sensitive *Staphylococcus aureus* colonization in NICU in patients stratified by Extreme Low Gestational Age Neonate groups (ELGAN= < 28 weeks).

Figure Footnote:

SA, *Staphylococcus aureus*

Table I. Characteristics of NICU patients in the study cohort admitted between November 2020-June 2022.

Study population (N= 383*)	**No. Infants (%) or Median (Q1-Q3)**
Sex (male)	199 (52.0)
Birth weight (gram)	1700 (1280-2365)
≤ 1000 gram	47 (12.3)
≤ 1500 gram	132 (34.5)
Gestational age (weeks)	32 (29-35)
ELGAN	48 (12.5)
Inborn	282 (73.6)
Cesarean section	235 (61.4)
Age at admission (days)	0 (0-3)

ELGAN; Extreme Low Gestational Age Neonates.

Table II. Comparison of characteristics of neonates colonized with MSSA with those not colonized with MSSA during the NICU stay, November 2020 – June 2022, Antwerp, Belgium; Univariate and multivariable analysis.

Variable	**Colonized (n= 164)** n (%), Median (IQR)	**Not colonized (n = 219)** n (%), Median (IQR)	**OR (95% CI)**	**Crude *P*-value**	**Adj. OR[a] (95% CI)**	***P*- value**
At admission						
Sex, (male)	82 (50.0)	117 (53.4)	0.87 (0.58-1.31)	0.536		
≤ 1000 gram	32 (19.5)	15 (6.8)	**3.30 (1.72-6.32)**	**0.0002***	**3.50 (1.21-10.7)**	**< 0.05***
ELGAN	36 (22.0)	12 (5.5)	**4.85 (2.43-9.67)**	**<0.0001***	**17.5 (4.34-102)**	**< 0.001*‡**
Vaginal delivery	72 (48.6)	92 (39.1)	1.47 (0.97-2.23)	0.0676	**5.41 (1.75 – 21.8)**	**< 0.01*‡**
Inborn	126 (76.8)	156 (71.2)	1.34 (0.84-2.13)	0.218		
During hospital stay						
CVC use	136 (82.9)	159 (72.6)	**1.83 (1.11-3.03)**	**0.0197***		
Intubation	46 (28.0)	58 (26.5)	1.08 (0.69-1.70)	0.8165		
NIV	130 (79.3)	163 (74.4)	1.31 (0.81-2.13)	0.2764		
Length of stay (days)**	11 (7-17)	13 (9-24)	**0.97 (0.95-0.98)**	**<0.0001***	**0.94 (0.92 – 0.96)**	**< 0.0001***

ELGAN, Extreme Low Gestational Age Neonates; CVC, Central Vascular Catheter; NIV, Non-Invasive Ventilation.

[a]Adjustment for confounders by stepwise logistic multivariable regression analysis: R-Square 0.13, Chi-Square 70, *P*-value < 0.001, ROC AUC 0.72

**P* < 0.05, ** # of days until the onset of infection or total stay for the infants without infection. ‡ Interaction term between ELGAN and Vaginal birth; $X^2 = 4.92 < P < 0.05$.

Table III. Comparison of characteristics of neonates with MSSA infection overall with those not infected with MSSA during the NICU stay, November 2020 – June 2022, Antwerp, Belgium; Univariate and multivariable analysis.

Variable	**MSSA Infected Overall (n= 49)** n (%), Median (IQR)	**Not MSSA Infected (n= 334)** n (%), Median (IQR)	**OR** (95% CI)	**Crude P-value**	**Adj. OR [a]** (95%CI)	**P- value**
At admission						
Sex, (male)	18 (36.7)	181 (54.2)	**0.49 (0.26-0.91)**	**0.0224***		
≤ 1000 gram	19 (38.8)	28 (8.4)	**6.291 (3.46-13.8)**	**<0.0001***	**8,32 (3,19-23,25)**	**< .0001**
ELGAN	20 (40.8)	28 (8.4)	**7.35 (3.78-15.01)**	**<0.0001***		
Vaginal delivery	20 (13.5)	29 (12.3)	1.10 (0.60-2.04)	0.737		
Inborn	42 (85.7)	240 (71.9)	**2.35 (1.01-5.41)**	**0.039***		
During hospital stay						
CVC use	42 (85.7)	253 (75.7)	**1.92 (0.83-4.44)**	**<0.0001***		
Intubation	18 (36.7)	86 (25.7)	1.67 (0.89-3.14)	0.106	**2,44 (1.033-5.84)**	**< .0001**
NIV	40 (81.6)	253 (75.7)	1.42 (0.66-3.05)	0.364		
Length of stay** (days)	23 (12-33)	15 (10-29)	1.004 (0.994-1.014)	0.416	**0.97 (0.95-0.98)**	**< 0.001**

MSSA colonization prior to infection	48 (98.0)	116 (34.7)	**90.2 (12.3-661)**	**<0.0001***	**27.49 (9.96-100.2)**	**<0.001***

MSSA, Methicillin Sensitive Staphylococcal aureus; IQR, Interquartile Range; OR, Odds Ratio, 95% CI, 95% Confidence Interval; ELGAN, Extreme Low Gestational Age Neonates (<28 weeks); CVC, Central Vascular Catheter; NIV, Non-Invasive Ventilation.

[a]Adjustment for confounders by stepwise logistic multivariable regression analysis : R^2 0.32, Chi-square 93.3 , *P*-value <0.001, ROC AUC 0.86.

*$P < 0.05$, ** # of days until the onset of infection or total stay for the infants without infection.

Table IV. Comparison of characteristics of neonates with invasive MSSA infection with those not infected with MSSA during the NICU stay, November 2020 – June 2022, Antwerp, Belgium.

Variable	**Invasive MSSA Infected (n= 11)** n (%), Median (IQR)	**Not MSSA Infected (n= 372)** n (%), Median (IQR)	**OR** (95% CI)	**Crude *P*-value**	**Adj. OR** [a] (95% CI)	**Adj. *P*-value**
At admission						
Sex, (male)	4 (36.4)	195 (52.4)	0.52 (0.15-1.80)	0.3657	0.19 (0.024-1.637)	0.13
≤ 1000 gram	7 (63.6)	40 (10.8)	**14.53 (4.07-51.80)**	**<0.0001**	2.01 (0.24-16.6)	0.51
ELGAN	7 (63.6)	41 (11.0)	**14.13 (3.97-50.34)**	**<0.0001**	**15.59 (1.39-174)**	**<0.05***
Vaginal delivery	4 (2.7)	7 (3.0)	0.90 (0.26-3.15)	1.0000	0.29 (0.03-2.57)	0.26
Inborn	11 (100.0)	271 (72.8)	N.D.	0.0743	N.D.	-
During hospital stay						
CVC use	11 (100.0)	284 (76.3)	N.D.	0.0755	N.D.	-
Intubation	10 (90.9)	94 (25.3)	**29.57 (3.74-234.11)**	**<0.0001**	**135 (7.8-2373)**	**<0.001***
NIV	9 (81.8)	284 (76.3)	1.39 (0.30-6.57)	1.0000	0.034 (0.029-4.05)	0.39
Length of stay (days)**	23 (9-16)	16 (10-29)	1.004 (0.986-1.022)	0.665	**0.95 (0.91-0.98)**	**< 0.05***
MSSA colonization prior infection	10 (90.9)	154 (41.4)	**14.16 (1.79-111.73)**	**0.0012**	**20.78 (1.92-224)**	**< 0.05***

MSSA, Methicillin Sensitive *Staphylococcal aureus;* IQR, Interquartile Range; OR, Odds Ratio, 95% CI, 95% Confidence Interval; ELGAN, Extreme Low Gestational Age Neonates (< 28 weeks); CVC, Central Vascular Catheter; NIV, Non-Invasive Ventilation; N.D., not determined.

[a]Adjustment for all covariates in univariate analysis by multivariable logistic regression analysis: R^2 0.59, Chi-square 59.4, *P*-value <0.0001, ROC AUC 0.98.

**P* < 0.05, ** # of days until the onset of infection or total stay for the infants without infection.

Table V. Performance of MSSA surveillance cultures for prediction of MSSA infection in NICU patients: effect of population, infection type and timing of cultures.

NICU Population	MSSA infection	Timing of cultures	Sensitivity [95% CI.]	Specificity [95% CI.]	PPV [95% CI.]	NPV [95% CI.]	Prevalence
Overall	**Overall**	1st week	**1.00**	**0.83**	0.25	**1.00**	5%
(N= 383)	*(N= 49)*		[0.77-1.00]	[0.77-0.87]	[0.15-0.39]	[0.98-1.00]	
		Beyond 1st	0.89	0.29	**0.29**	0.89	**24%**
		week	[0.75-0.96]	[0.23-0.39]	[0.21-0.38]	[0.76-0.96]	
		All weeks	0.98	0.65	0.29	0.99	12.8%
			[0.89-0.99]	[0.6-0.70]	[0.22-0.36]	[0.97-0.99]	
	Invasive	1st week	**1.00**	**0.79**	0.04	**1.00**	0.09%
	(N= 11)		[0.34-1.00]	[0.73-83]	[0.01-0.13]	[0.98-1.00]	
		Beyond 1st	0.88	0.24	**0.07**	0.97	**6.1%**
		week	[0.56-0.98]	[0.18-0.32]	[0.04-0.13]	[0.85-0.99]	
		All weeks	0.90	0.58	0.06	0.99	2.9%
			[0.62-0.98]	[0.53-0.63]	[0.03-0.11]	[0.97-0.99]	
ELGAN	**Overall**	1st week	**1.00**	**0.76**	0.50	**1.00**	18.8%
(N= 48)	*(N= 20)*		[0.43-1.00]	[0.49-0.91]	[0.18-0.81]	[0.72-1.00]	
		Beyond 1st	1.00	0.13	**0.56**	1.00	**53.1%**
		week	[0.81-1.00]	[0.04-0.37]	[0.39-.72]	[0.34-1.00]	
		All weeks	1.00	0.42	0.55	1.00	41.7%
			[0.83-1.00]	[0.26-0.61]	[0.39-0.70]	[0.75-1.00]	
	Invasive	1st week	**1.00**	**0.66**	0.17	**1.00**	6.3%
	(N= 7)		[0.20-1.00]	[0.41-0.84]	[0.03-0.56]	[0.72-1.00]	
		Beyond 1st	1.00	0.07	**0.20**	1.00	**18.8%**
		week	[0.61-1.00]	[0.02-0.24]	[0.09-0.37]	[0.34-1.00]	
		All weeks	1.00	0.32	0.19	1.00	14.6%
			[0.64-1.00]	[0.19-0.47]	[0.1-0.36]	[0.77-1.00]	

[95% CI.], 95% Confidence Interval; PPV, Positive Predictive Value; NPV, Negative Predictive Value