Trends, risk factors and outcomes of healthcare-associated infections in a neonatal intensive care unit in Italy during 2013–2017

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Abstract

Background: Healthcare-associated infections (HAIs) occur frequently in intensive care units (NICUs). The aim of this study was to analyze the results of surveillance of HAIs in a III level NICU in Naples, Italy during 2013–2017 and to compare with those obtained during 2006–2010.

Methods: The surveillance included 1265 neonates of all birth weight (BW) classes with > 2 days NICU stay. Infections were defined using standard Centers for Disease Control and Prevention definitions adapted to neonatal pathology.

Results: A total of 125 HAIs were registered during 2013–2017 with a frequency of 9.9% and an incidence density of 3.2 per 1000 patient days. HAIs occurred in all BW classes with a decreasing trend from the lowest to the highest BW classes (p = < 0.001). Central line-associated blood stream infection (CLABSI) was the most frequent infection (69.6%), followed by ventilator associated pneumonia (VAP) (20%), urinary tract infection (UTI) (8.8%) and necrotizing enterocolitis (NEC) (1.6%). Also, CLABSI and VAP incidence density decreased from lower to highest BW classes showing a significant trend (p = 0.007). Most frequent pathogens responsible for CLABSI were: Coagulase-negative staphylococci (CONS) (25.3%), Candida parapsilosis (21.8%), Pseudomonas aeruginosa (5.7), Escherichia coli and Klebsiella pneumoniae (6.8%). No microbiological diagnosis was achieved for 20.7% of CLABSI. Pseudomonas aeruginosa (28%), Stenotrophomonas maltophilia (20%), and CONS (20%) were the most frequent pathogens responsible for VAP. CLABSI incidence density showed no differences between 2006 and 2010 and 2013–2017, while VAP incidence density for the 751–100 g BW class was higher during 2006–2010 than during 2013–2017 (p = 0.006). A higher incidence of the CLABSI caused by Gram positive bacteria (p = 0.002) or by undetermined etiology (p = 0.01) was observed during 2013–2017 than during 2006–2010, while a significant lower incidence of VAP caused by Gram-negative bacteria was found during 2013–2017 than during 2006–2010 (p = 0.007).

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Background
Healthcare-associated infections (HAIs) occur frequently in neonates admitted to intensive care units and recognizes many risk factors, including low birth weight and factors related to invasive procedures, such as vascular catheterization and mechanical ventilation [1, 2].

Active infection surveillance is recognized internationally as one of the activities that contribute to the reduction of the incidence of infections in hospitalized neonates [3–12]. Several active surveillance protocols for HAIs in neonatal intensive care units (NICUs) have been established worldwide, who differs in type of infections/outcomes and/or patients’ population included in the surveillance. In particular, the neonIN network in UK, the Vermont Oxford Network in USA and the Canadian Neonatal Network HAIs surveillance protocols focus mainly on early- and late-onset sepsis, while the NHSN and the NEO-KISS protocols extend surveillance of HAIs to VAPs and HAPs [10, 11]. Also, the majority of HAIs surveillance systems includes all patients’ population of NICUs [4–6, 8, 9, 12], while the NEO-KISS system monitors HAIs in infants of BW < 1500 g in NICUs until discharge, until death, or until they reach 1800 g [10, 11]. Moreover, benchmarking of HAIs surveillance protocols in NICUs is available on a national scale for NHSN [4–6], NEO-KISS [10, 11], the Vermont Oxford Network [7, 8], the neonIN network [12], and the Canadian Neonatal network [9]. A national framework for the surveillance of HAIs in Italy-SPIN-UTI project is active in Italy since 2008, which involves adult Intensive care units (ICUs) with none of NICUs [13, 14]. Although no surveillance network for HAIs in NICUs is present in Italy, few studies reported the prevalence [15], incidence of HAIs [16–20] and epidemics of specific nosocomial pathogens [20–24] in selected NICUs.

We recently analyzed the results of HAIs surveillance in a III level NICU in Naples, Italy during 2006–2010 using the NHSN surveillance protocol [18]. Our data showed that HAIs developed in all BW classes, but low BW neonates were at major risk to acquire HAIs [18]. During the surveillance, we observed an outbreak of extensively drug-resistant (XDR) Acinetobacter baumannii in the NICU from November 2010 to July 2011, which required the temporary closure of the ward to external admissions of neonates during 2011 [21]. To better understand the risk factors of infection and the impact of prevention activities, we decided to expand our data and compare the results of surveillance of HAIs in the NICU using the NHSN surveillance protocol during 2013–2017 and to compare HAIs with those obtained during 2006–2010.

Methods
Setting
The NICU of the University Hospital of Naples “Federico II”, Italy is a III level Unit with a total of 25 incubators and cradles, 8 for intensive care and 17 for intermediate care. The ward serves the University Obstetric Clinic (approximately 2000 births/year) which is both a high-risk pregnancy center and an obstetric emergency service. Moreover, outborn neonates from the regional Newborn Emergency Transport Service are hospitalized in the NICU. In 2013–2017 years, a median (min, max) number of infants per year equal to 280 (246, 311) was admitted.

Active surveillance
Healthcare-associated infections (HAIs) active patient-based surveillance (AS) is the identification of HAIs by trained personnel who proactively look for HAIs using multiple data sources. Data are collected from the medical records of the patients every week. The surveillance personnel conduct rounds on the ward to review the results from the laboratory and sometimes from the radiology findings and to look on the medical records for signs and symptoms of infection, according to the standard definitions. Any clinical issues are directly discussed with caregivers. Data are analyzed on monthly basis and expressed as monthly report. Monthly report consists of patient data, data on swab isolations of sentinel pathogens, device utilization ratios and infection data. All neonates with > 2 days NICU stay enter the AS system and data regarding date of birth, birth weight (BW), gestational age, type of delivery, Apgar score, date of admission in the ward, date of infection, discharge date, type of microorganisms isolated, use of invasive devices (days of central line catheterization, including umbilical catheterization, and invasive ventilation), antimicrobial
therapy exposure and infections are collected. The end
of the surveillance period coincides with the discharge of
the newborn from the ward. Infections are defined using
standard Centers for Disease Control and Prevention
(CDC) definitions adapted to neonatal pathology [3] and
are considered to be health care associated if they de-
velop >2 days after NICU admission. VAP was defined
using CDC criteria for defining nosocomial pneumonia
for infants ≤1 year old [25]. We exclude neonates with
congenital or perinatal infection. This paper analyzes
data from the HAIs AS system over a 5 years period
(2013–2017). For this study purposes, only CLABSI,
pneumonia, necrotizing enterocolitis (NEC), and urinary
tract infections (UTIs) were considered. Central line-
associated blood-stream infections (CLABSI) and
ventilator-associated pneumonia (VAP) were attributed
if a central line, including umbilical, catheter and inva-
sive ventilation, respectively, were in place at the time of
or within 48 h prior to the development of the infection.
The etiology of all infections within each BW class was
assessed during the study period. HAIs surveillance
was regulated by the Regional Health Authority and it was
one of the basic components of the Regional Plan for
Healthcare-associated infections Prevention and Control
[26]. Results of surveillance activities were periodically
reported to the Regional Health Authority.

Statistical analysis
Descriptive data of device-associated infections were
expressed as absolute number, percentage and incidence
densities (presented per 1000 specific device-days) with
respective 95% confidence intervals (CIs). If a neonate had
multiple episodes of HAIs, each episode was considered as
an independent event. The total number of device-associated
infections and the total days of device utilization were com-
puted for each birth weight class. Frequency measures
were calculated as percent of infection and as incidence
densities, i.e. infection rates per 1000 patient days or 1000 days of
directly related invasive device within 5 BW categories (≤750 g,
751–1000 g, 1001–1500 g, 1501–2500 g, and ≥2501 g). De-
vice utilization rates within such classes were also calculated.
To detect whether a significant birth weight trend occurred,
we fitted a generalized linear model with a Poisson link func-
tion. The log transformed total device-days value was used as
an offset in the model. Separate models were fitted for
device-associated CLABSI, and VAPs.

To compare the second study period with those ob-
tained during 2006–2010 study period, we included in
the models an interaction terms (birth weight*study
period) to explore whether there were differences in
HAI density between the two study periods by birth
weight class.

Generalized linear model with a Poisson link function
was performed also to test for differences among
etiology of device-associated infections, using gram neg-
avatives as reference group. To compare the two study pe-
riods also in terms of etiology of device-associated
infections, the interaction terms (etiology *study period)
was included in the models. Separate models were fitted
for CLABSI and VAPs. All statistical analyses were per-
formed using the R software environment for statistical
computing, version 3.6.0 [27]. For all statistical analysis,
a $p$-value<0.05 was considered as statistical significance.

Results
During 2013–2017, 1265 neonates, corresponding to
90.68% of all admissions to the ward, entered the HAIs
AS system with a total of 39,207 days of stay, 12,140 days
of use of central-line catheter and 7591 days of mechan-
ical ventilation (Table 1). The remaining 9.32% of admis-
sions was excluded because either ineligible (length of
stay <2 days) or missed by the HAIs AS system. During
the study period, neonates with >1000 g BW accounted
for 65.86% of total patient days (1001–1500 g, 1501–
2500 g, and ≥2501 g classes representing 23.6, 28.7, and
13.6%, respectively). Neonates with extremely low BW
(ELBW) (≤750 g and 751–1000 g BW classes) were
34.14% of patient days in the NICU accounting for 16.1
and 18.04% of total patient days, respectively).

The overall number of HAIs registered during the
study period by the local AS system was 125 (which cor-
responded to a total infection rate of 9.9% and a total in-
cidence density of 3.2 per 1000 patient days). The crude
mortality rate of the patients under surveillance was
5.45%. The mortality rate of the infected patients was
19.4%. HAIs developed in all BW classes, but 60.8% of
all HAIs developed in patients of ≤1000 g weight at
birth. Also, a significant decreasing trend of incidence
density of HAIs per 1000 patient days was observed
from the lowest to the highest BW classes (7.44, 4.1,
2.16, 2.13 and 0.94 in ≤750 g, 751–1000 g, 1001–1500 g,
1501–2500 g, and ≥2501 g BW newborns, respectively,
$p < 0.001$) (Fig. 1). CLABSIs proved to be the most fre-
quenct infections (69.6%), followed by VAPs (20%), UTIs
(8.8%), and necrotizing enterocolitis (NECs) (1.6%). De-
vice associated infections (i.e. CLABSIs and VAPs) rep-
resented 89.6% of HAIs. A significant decreasing trend
from the lowest to the highest BW classes ($p = 0.007$)
was found for incidence densities of device associated
infections during 2013–2017 study period (Fig. 2). Inci-
dence densities of CLABSIs and VAPs across the five
BW classes are shown in Table 2. The incidence density
of CLABSIs decreased significantly from the lowest to
the highest BW classes ($p = 0.001$), while the incidence
density of the VAPs decreased in the first three classes
of BW and increased in the 1501–2500 g and ≥2501 g
BW classes, showing a non-significant trend ($p = 0.174$).
Table 1 Neonates data per birthweight category during the study period

| Year | 2013 | 2014 | 2015 | 2016 | 2017 | Total |
|------|------|------|------|------|------|-------|
| Hospitalized patients | 246 | 274 | 311 | 280 | 284 | 1395 |
| Surveilled patients | 232 | 248 | 287 | 254 | 244 | 1265 |
| Number of patient days<sup>a</sup> | 8186 | 7598 | 7707 | 7604 | 8112 | 39,207 |
| ≤ 750 g | 1583 | 990 | 1083 | 1502 | 1156 | 6314 |
| 751–1000 g | 1753 | 2064 | 1328 | 820 | 1108 | 7073 |
| 1001–1500 g | 1654 | 1666 | 1557 | 1897 | 2476 | 9250 |
| 1501–2500 g | 2043 | 2035 | 2566 | 2385 | 2222 | 11,251 |
| > 2500 g | 1153 | 843 | 1173 | 1000 | 1150 | 5319 |
| Number of central line days<sup>a</sup> | 2442 | 2190 | 2549 | 2479 | 2480 | 12,140 |
| ≤ 750 g | 577 | 477 | 519 | 695 | 405 | 2673 |
| 751–1000 g | 629 | 725 | 551 | 292 | 336 | 2533 |
| 1001–1500 g | 503 | 422 | 557 | 620 | 929 | 3031 |
| 1501–2500 g | 490 | 383 | 637 | 704 | 610 | 2824 |
| > 2500 g | 243 | 183 | 285 | 168 | 200 | 1079 |
| Number of ventilator days<sup>a</sup> | 2018 | 932 | 1141 | 1774 | 1726 | 7591 |
| ≤ 750 g | 897 | 323 | 407 | 826 | 506 | 2959 |
| 751–1000 g | 529 | 371 | 406 | 220 | 341 | 1867 |
| 1001–1500 g | 228 | 89 | 134 | 300 | 523 | 1274 |
| 1501–2500 g | 280 | 75 | 90 | 363 | 294 | 1102 |
| > 2500 g | 84 | 74 | 104 | 65 | 62 | 389 |

<sup>a</sup>Data refer to cases per 1000 specific device-days

Fig. 1 Trend of HAIs incidence densities (95% CI) per 1000 patient days across BW categories. P-values are obtained from Poisson Regression for testing whether incidence densities significantly vary across BW categories

Fig. 2 Trend of incidence densities (95% CI) of device-associated infections per 1000 days of device utilization across BW categories. P-values are obtained using Poisson Regression for testing whether incidence densities significantly vary across BW categories
Aetiology of device-associated infections among the five BW classes is shown in Table 3. Most frequent pathogens responsible for CLABSIs were: Coagulase-negative staphylococci (CONS) (25.3%), Candida parapsilosis (21.8%), Candida albicans (4.6%), Pseudomonas aeruginosa (5.7%), Escherichia coli and Klebsiella pneumoniae (6.8%). No microbiological diagnosis was achieved for 20.7% of CLABSIs. P. aeruginosa (28%), Stenotrophomonas maltophilia (20%), and CONS (20%) were the most frequent pathogens responsible for VAP. During the study period, the incidence density of Gram-positive CLABSI was significantly higher than the incidence density of Gram-negative CLABSI (2.47 and 1.40, respectively) (p = 0.008). On the other hand, the incidence density of VAPs caused by Gram negative was significantly higher than that caused by Gram-positive (2.11 and 0.26, respectively) (p < 0.001) and by polymicrobial aetiology (2.11 and 0.92, respectively) (p = 0.001). No microbiological diagnosis was achieved in 16% of infections.

The most frequent device-unrelated HAIs were UTIs (8.8% of all), which mainly affected neonates belonging to the 751–1000 g and 1501–2500 g BW classes (distribution of UTIs was 0, 54.5, 9.1, 27.3 and 9.1% in ≤750 g, 751–1000 g, 1001–1500 g, 1501–2500 g and >2500 g BW classes, respectively).

### Table 2 Incidence densities of device-associated infections per birth weight category in surveilled neonates

| Device-associated infection | ≤ 750 g | 751–1000 g | 1001–1500 g | 1501–2500 g | > 2500 g | p-value |
|----------------------------|---------|------------|-------------|-------------|---------|---------|
| CLABSI                     | 11,972  | 7106       | 5609        | 6020        | 2780    | 0.001   |
| VAP                        | 5069    | 2142       | 0.785       | 3630        | 2571    | 0.174   |

**Abbreviations:** CLABSI: central line-associated bloodstream infection, VAP: ventilator-associated pneumonia

### Table 3 Etiology of device-associated infections per birth weight category in surveilled neonates

| CLABSI                          | ≤750 g | 751–1000 g | 1001–1500 g | 1501–2500 g | > 2500 g | Total (%) |
|---------------------------------|--------|------------|-------------|-------------|---------|-----------|
| CONS                            | 8      | 4          | 5           | 4           | 1       | 22 (25.3) |
| Candida parapsilosis            | 7      | 3          | 6           | 2           | 1       | 19 (21.8) |
| Not determined                  | 8      | 3          | 3           | 4           |         | 18 (20.7) |
| Pseudomonas aeruginosa          | 2      | 2          | 1           |             |         | 5 (5.7)   |
| Candida albicans                | 1      | 2          | 1           |             |         | 4 (4.6)   |
| Escherichia coli                | 1      | 1          | 1           |             |         | 3 (3.4)   |
| Escherichia coli ESBL+          | 2      | 1          |             |             |         | 3 (3.4)   |
| Klebsiella pneumoniae           | 2      |            |             |             |         | 3 (3.4)   |
| Klebsiella pneumoniae ESBL+     |        |            |             |             |         |           |
| Staphylococcus aureus           | 1      | 1          |             |             |         | 2 (2.3)   |
| Enterococcus faecalis           |        |            |             |             |         |           |
| Streptococcus sanguinis         |        |            |             |             |         |           |
| Kocuria kristinae               |        |            |             |             |         |           |
| Candida pelliculosa             |        |            |             |             |         |           |
| Total (%) within BW category    | 32 (36.8) | 18 (20.7) | 17 (19.5) | 17 (19.5) | 3 (3.4) | 87 (100) |

| VAP                            | ≤750 g | 751–1000 g | 1001–1500 g | 1501–2500 g | > 2500 g | Total (%) |
|--------------------------------|--------|------------|-------------|-------------|---------|-----------|
| Pseudomonas aeruginosa         | 4      | 1          | 2           |             |         | 7 (28)    |
| CONS                           | 4      | 1          |             |             |         | 5 (20)    |
| Stenotrophomonas maltophilia   | 4      | 1          |             |             |         | 5 (20)    |
| Staphylococcus aureus          | 1      | 1          |             |             |         | 2 (8)     |
| Acinetobacter baumannii        | 1      | 1          |             |             |         | 2 (8)     |
| Enterobacter cloacae           |        |            |             |             |         |           |
| Klebsiella pneumoniae ESBL+    |        |            |             |             |         |           |
| Total (%) within BW category   | 15 (60) | 4 (16)     | 1 (4)       | 4 (16)      | 1 (4)   | 25 (100)  |
751–1000 g, 1001–1500 g, 1501–2500 g, and ≥ 2501 g BW newborns, respectively. Fifty-five percent of UTIs were caused by *E. coli* (18.18%), *K. pneumoniae* (18.18%) and *Enterobacter spp.* (18.18%), the remaining 45.5% were caused by *Klebsiella oxytoca*, *Enterococcus faecalis* and polymicrobial aetiologies. In the 2013–2017 study period, we had two cases of Necrotizing Enterocolitis (NEC) and no aetiology was defined in both of cases.

Then, a comparison was performed between 2013 and 2017 study period and 2006–2010 study period [18]. The number of patient days was 43,447 in the first period and 39,207 in the second period, the number of central line utilization days was 4232 in the first period and 12,140 in the second period. Table 4 shows the distribution of patient days and devices utilization across BW classes during the two study periods. The number of patient days, central line utilization and ventilation utilization increased by 1.5-fold in the ≤750 g BW class during 2013–2017 study period, while percentages of devices utilization decreased in the > 2500 g BW class during 2013–2017 study period. No other relevant differences were found between the two study periods (Table 4).

Incidence density of device associated infections per 1000 days of catheter or ventilation utilization during 2013–2017 were then compared to the incidence density obtained during 2006–2010 [18]. No significant differences were found between the two periods (6.17 for the first period vs. 5.67 for the second period, *p* = 0.548). Furthermore, no significant differences between the two periods were found when CLABSI incidence densities per 1000 catheter days were compared between the two periods (5.58 for the first period vs. 7.16 for the second period, *p* = 0.206). In contrast, VAP incidence densities per 1000 days of ventilation utilization were significantly different between the two periods (6.91 for the first period vs. 3.29 for the second period, *p* = 0.004).

Finally, the incidence densities of CLABSI per 1000 catheter days by birth weight class and incidence densities of VAPs per 1000 days of ventilation utilization by birth weight class during 2013–2017 were compared to device-associated infection incidence densities during 2006–2010. There were no significant differences between the two periods for CLABSI, whose incidence densities showed a decreasing trend from the lowest to the highest BW classes in both study periods (Fig. 3a). On the other hand, the incidence of VAPs in the 751–1000 g BW class was significantly higher during 2006–2010 than 2013–2017 study period (*p* = 0.006) (Fig. 3b). Moreover, a significant higher incidence density of CLABSI caused by Gram-positive bacteria (*p* = 0.002) and by undetermined aetiologies (*p* = 0.009), and a significant lower incidence of VAPs caused by Gram-negative bacteria (*p* = 0.007) was found during 2013–2017 compared to the 2006–2010 study period (Fig. 4a and b, respectively). The crude mortality rate of the patients under surveillance in the 2006–2010 study period was 4.83%. The mortality rate of the infected patients was 14.9%. Both the crude mortality rate and the mortality rate of infected patients increased during 2013–2017 compared with the 2006–2010 study period, being 5.45% vs 4.83 and 19.4% vs 14.9%, respectively.

### Discussion

HAIs are frequent complications occurring during hospitalization of newborns in NICUs and are associated with patients’ susceptibility conditions such as prematurity and immune status, use of invasive devices, such as central and/or umbilical vascular catheterization and mechanical ventilation, total parenteral nutrition, antimicrobial use and other concomitant drugs therapeutic variables [1, 2]. We recently reported the results of the surveillance of HAIs in a NICU in Italy using the NHSN surveillance protocol [18].

In the present study, we analyzed the results of surveillance of HAIs during 2013–2017 and compared them with HAIs during 2006–2010 in the same NICU [18] as internal benchmarking. During 2013–2017, total infection rate and total incidence density per

### Table 4

| Patient days | ≤ 750 g | 751–1000 g | 1001–1500 g | 1501–2500 g | > 2500 g |
|-------------|--------|------------|-------------|-------------|---------|
| 2006–2010   | 9%     | 17%        | 27%         | 28%         | 19%     |
| 2013–2017   | 16%    | 18%        | 24%         | 29%         | 13%     |

| Central line utilization | ≤ 750 g | 751–1000 g | 1001–1500 g | 1501–2500 g | > 2500 g |
|--------------------------|---------|------------|-------------|-------------|---------|
| 2006–2010                | 13%     | 21%        | 29%         | 22%         | 15%     |
| 2013–2017                | 22%     | 21%        | 25%         | 23%         | 9%      |

| Ventilation utilization | ≤ 750 g | 751–1000 g | 1001–1500 g | 1501–2500 g | > 2500 g |
|-------------------------|---------|------------|-------------|-------------|---------|
| 2006–2010               | 26%     | 31%        | 18%         | 15%         | 10%     |
| 2013–2017               | 39%     | 25%        | 17%         | 14%         | 5%      |
1000 patient days of HAIs were similar to those found during 2006–2010 (9.9% and 3.2 vs. 9% and 3.5). In partial accordance with our data, a single center cohort study in an Italian NICU reported an infection rate of 13.2% and an incidence density of 7.8 HAIs per 1000 patient days [16] and a multicenter prospective cohort study in six Italian NICUs reported an infection rate of 12.8% and an incidence density of 6.93 HAIs per 1000 patient-days [17]. Also, a multicenter retrospective cohort study in pediatric intensive care units (PICUs) and NICUs in Italy and Brazil during 2010–2014 described a cumulative incidence of HAI of 3.6/100 ICU admissions and an infection rate of 3.6/1000 ICU days [19].

Similarly to HAIs occurred during 2006–2010 study period in the same NICU [18], HAIs were more frequent in low birth weight groups (< 1000 g 60.8%; in detail BW1 < 750 g 37.6% and BW2 750-1000 g 23.2%) but developed in all BW classes. This reinforces the need to surveil all BW classes in NICUs according to NHSN protocol [3–6] and not infants of BW ≤ 1500 g according to NEO-KISS protocol [10, 11].

During 2013–2017 study period, device associated infections, i.e. CLABSIs (69.6%) and VAPs (20%), represented 89.6% of HAIs in our NICU. Incidence density of device associated infections did not significantly change between the two study periods (6.17 for the first period vs. 5.67 for the second period, \( p = 0.548 \)). An increase of
CLABSI incidence densities per 1000 catheter days, although not significant, was observed during 2013–2017 period respect to 2006–2010 period (7.16 versus 5.58 \( p = 0.206 \)), which can be dependent by the increase in the number of patient days and central line utilization in the \( \leq 750 \text{ g BW} \) class in the second study period (Table 4). This finding is in agreement with previous studies showing that bloodstream infections prevailed among HAIs in Italian NICUs [16, 17] and that bloodstream infections were the main infections (45.4%), followed by lower respiratory tract infections (27.8%) and urinary tract infections (15.8%) in Italian and Brazilian PICUs and NICUs [19]. High percentage of bloodstream infections in NICUs has been also reported by NHSN in USA [4], neonlNnetwork in UK [12] and worldwide [28–31].

Finally, the increase of CLABSI incidence density during 2013–2017 in our NICU is alarming because CLABSI rates measure Hospital performance for high-quality patient care [32].

On the other hand, a significant increase of VAP incidence density in our NICU was found for the 751–1000 g BW class during 2006–2010 compared with 2013–2017 period (Fig. 3b). This might have been dependent on the increase in VAPs in very-low birth weight neonates caused by two outbreaks during 2006–2010 period in the NICU [21, 24]. The elevated number of device-associated infections in the NICU strengthen the importance to calculate device utilization rates and use as risk factors for the development of CLABSIs and VAPs according to NHSN surveillance protocol [3–5]. Also, in agreement with previous surveillance studies of HAIs in other NICUs [16, 19, 29] and in the same NICU during 2006–2010 [18], UTIs were the third most frequent cause of HAIs after CLABSIs and VAPs in our NICU, but decreased from 28.8% [18] to 8.8% during 2013–2017.

Both the crude mortality rate and the mortality rate of infected patients increased during 2013–2017 compared with the 2006–2010 study period. This could have been contributed by the higher number of patient days, central line utilization and ventilation utilization in the \( \leq 750 \text{ g BW} \) class and the higher number of CLABSI during 2013–2017 compared with 2006–2010 study period.

Additional epidemiological information was provided by the analysis of the incidence densities of etiology of CLABSIs and VAPs in our NICU during 2013–2017 and the comparison with those found during 2006–2010. During 2013–2017, Gram-positive bacteria, in particular CONS (25.3%) and \( S. \) aureus (2.3%), were the most frequent pathogens responsible for CLABSIs. This finding is in accordance with several reports showing that gram-positive bacteria are the main cause of bloodstream infections in neonates in the NICUs [4, 12, 17, 28–30]. Other most frequent pathogens responsible for CLABSIs in the NICU were \( \text{Candida spp.} \), \( C. \) parapsilosis (21.8%) and \( C. \) albicans (4.6%), \( P. \) aeruginosa (5.7%), \( E. \) coli (3.4%) and \( K. \) pneumoniae (3.4%). During 2013–2017, VAPs were most frequently caused by \( P. \) aeruginosa (28%), \( S. \) maltophilia (20%), and CONS (20%). UTIs represented the most frequent device-unrelated infection in the NICU during 2013–2017 and were most frequently caused by \( E. \) coli (18.18%), \( K. \) pneumoniae (18.18%) and \( Enterobacter \) spp. (18.18%). The comparison of the etiologies of CLABSIs in the NICU between the two study periods showed a significant higher incidence density of CLABSIs caused by Gram-positive bacteria \( (p = 0.002) \) and by undetermined etiology \( (p = 0.01) \) and a significant lower incidence of CLABSIs caused by \( \text{Candida spp.} \) \( (p = 0.05) \) during 2013–2017 compared to 2006–2010 period. Moreover, a significant lower incidence of VAPs caused by Gram-negative bacteria \( (p = 0.007) \) were found during 2013–2017 comparing to 2006–2010 period. The differences in the etiologies of VAPs and CLABSIs between the two periods could have been due to the occurrence of \( P. \) aeruginosa and \( A. \) baumannii outbreaks in the NICU during the first study period [21, 24]. Moreover, an increase in bloodstream infections caused by \( C. \) parapsilosis was observed during 2009–2012 in the NICU [22], which might have been responsible for the high incidence of CLABSIs caused by \( \text{Candida spp.} \). The increase of CLABSIs by undetermined etiology during 2013–2017 is worrying and without a definite cause.

We recognize that our study has limitations that affect the generalization of our results. The first limitation relies on the retrospective nature of the study, which did not allow to evaluate the efficacy of specific infection control measures to prevent HAIs in the NICU. One other limitation relies on the single center nature of the study, which did not allow to compare results among different clinical settings and to create a benchmarking on a national scale. Additional limitation of the study was the lack of analysis of inborn and outborn status, total parenteral nutrition, antimicrobial use and other concomitant drugs therapeutic variables of neonates included in the study. Future studies will be necessary to investigate the above issues.

**Conclusion**

HAIs in our NICU during 2013–2017 developed in all BW classes with a decreasing trend from the lowest to the highest BW classes. CLABSIs, VAPs and UTIs were the most frequent HAIs. The use of central line catheter and mechanical ventilation invasive devices was associated with high risk of HAIs in our NICU. An increase of CLABSI incidence densities per 1000 catheter days, although not significant, was observed during 2013–2017 period respect to 2006–2010 period. Also, an higher incidence of the CLABSIs caused by Gram-positive bacteria or by undetermined etiology and a lower incidence
of VAPs caused by Gram-negative bacteria were found during 2013–2017 study period respect to 2006–2010 period. This reinforces the importance of device associated HAIs surveillance protocol in the NICU, which monitors microbiological isolates responsible for infection and use of central line and assisted ventilation in all BW classes of neonates.

Abbreviations
AS: Active surveillance; AV: Assisted ventilation; BSI: Bloodstream infection; BW Birth weight; CLABSI: Central line-associated bloodstream infection; CVC: Central venous catheter; ELBW: Extremely low birth weight; HAIs: Healthcare-associated infections; NICU: Neonatal intensive care; VAP: Ventilator associated pneumonia

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Authors’ contributions
MSS, FR and RZ collected the clinical and epidemiological data from patient’s charts. MSS, PD EPE and RZ analyzed and interpreted the data. MT and RZ conceived the study and participated in its design and coordination. PD performed the statistical analysis. MSS, PD and RZ wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The dataset supporting the conclusions of this article will be made available from the corresponding author upon request.

Ethics approval and consent to participate
The study was approved by the Ethics committee of the Federico II University Hospital (protocol number 173/2017). No written informed consent was necessary for this type of study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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