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Incidence and factors associated with nosocomial infections in a neonatal intensive care unit (NICU) of an urban children's hospital in China

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Summary

Objective: The study's aim was to assess incidence and epidemiologic profile of nosocomial infection (NI) in a NICU of China, and to identify main risk factors of NIs. *Materials and Meth*ods: Chi square test for discrete variables and independent t-test for continuous variables to examine the association with NI. Univariate regression model was applied to the variables to predict the NI status. Finally the multivariate model was utilized with stepwise methods included all variables in the univariate model to extrapolate the independent variables to NI. *Results:* Infection rate in NICU during the study period was 6.2 episodes per 100 patients. Infection density was 4.2 episodes per 1,000 patient-days. Infection rate of ventilation-related pneumonia was 3.4 episodes per 1,000 mechanical ventilation (MV) days. Central line-associated bloodstream infection rate was 5.4 episodes per 1,000 central line days. Gestational age < 32 weeks, with congenital malformation, twins or triplets, gastric tube feeding, operation, duration of prophylaxis antibiotic use, duration of probiotic use, duration of parenteral nutrition were the risk factors associated with NI in NICU. We discussed the association between intrinsic factors of infants and health care procedures with NI. *Conclusion:* This study provided information for prevention strategies of NI, that will ultimately improve the healthcare service level.

Key words: Nosocomial infection; Neonatal intensive care unit; Risk factors; Incidence.

Introduction

Nosocomial infection (NI) refers to healthcare associated infection that was recognized over one century ago. Obstetrics and Gynecology doctors, Ignac Philipp Semmel-Weis observed that the mortality rate of women delivering and of the newborns could be highly reduced through hand disinfection of midwives [1]. Nowadays healthcare-associated infection has become a severe problem worldwide, which takes a high death toll in human lives and affects hundreds of millions of patients each year [2].

Neonatal intensive care unit (NICU) patients, including preterm babies and infants who require surgeries are unique and highly vulnerable group of people. The research indicated that the NI rate was on the rise in the past decade, due to the increasing survival rate of early preterm infants and the extensive use of invasive healthcare procedures [3]. The incidence of NI in NICU varied from 6%-40%, which depended on the proportion of preterm infants and the proportion of surgery in NICU, which was much higher than the incidence rate of normal neonatal wards, which varied from 0.3 - 1.7% [4].

Another key factor which is influencing the prevalence of NI in NICU is socio-economic level of the nation. The situation is much worse in developing countries than in developed countries such as USA and European countries. A recent review from WHO found that prevalence of healthcare-associated infection which was 15.5 per 100 patients in developing countries, which was much higher than the prevalence reported from Europe and the USA. Pooled overall healthcare-associated infection density in adult intensive-care units was 47.9 per 1,000 patient-days (95% CI 36·7~59·1), at least three times as high as incidences reported in the USA [5].

The outcome associated with the NI was the increasing mortality and morbidity, as well as the prolonged hospital stay. In the USA, the extension of hospital stay and the additional treatment for healthcare-associated infection cost the government 17 to 29 billion dollars [3]. The degree of prematurity, birth weight, applied mechanical ventilation (MV) and exposure to central venous catheter were the risk factors reported in previous articles [6].

There was increasing trends in the past decades of NI in China. According to the published articles, the infection rate of NICU in China varied from 7% - 11.6% [7,8]. However, the risk factors, such as length of hospitalization, applied gastric tube, and applied ventilation associated with the NI in NICU in China are rarely reviewed and analyzed [9]; most of the studies only discuss the epidemiology profile of NI in NICU [8, 10, 11]. Since China's healthcare system's regulation procedure, efficiency, and socio-economic situa-

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tions are unique, it is absolutely essential to conduct the research on the NI infection incidence and risk factors in order to control and minimize the influence. This study aimed to assess the incidence and epidemiologic profile of NI in NICU of China, and to identify the main risk factors of NIs.

Materials and Methods

Setting

The study was conducted in the NICU of Guangzhou Women and Children's Medical Center. There were 30 cots in NICU, with three m² surface area for each cot and one m distance between two cots. The ratio of nurse-to-cot was 1:4. The entire area was separated into two parts, one for the non-infectious patients and one for infectious patients. The NICU was equipped with air filters. There are about 800 patients admitted in the NICU per year. Annual patient days are around 11,000. There are about 16% of the patients admitted for surgery and 60% preterm birth among all admitted patients during the study period. The present authors accepted patients aged mainly within 28 days, but if the infants were preterm and the weight was not heavier than 4,000 g, this rule was not applied. All the NI cases were informed by the attending physicians and confirmed by the associate chief doctor or chief doctor and the experienced infection control staff.

Inclusion and exclusion criteria

This study was a retrospective cohort survey with a nested case control study. All 1,653 Chinese infants admitted in the NICU during the study period from December, 2009 to May, 2012 were included as the subjects.

All Chinese infants admitted and diagnosed with at least one episode of NI in the NICU of Guangzhou Women and Children's Medical Center from December of 2009 to May of 2012 were included as the cases. There were 81 infants with 103 episodes in total. Meanwhile 300 controls were randomly sampled based on the computerized records of all infants admitted and stayed in NICU for more than 48 hours without infection in the hospital in the same period.

The infants who died (n=8) or were discharged or transferred to other department within 48 hours (n=23) after being admitted in NICU were excluded. The subjects with missing data in more than two variables (n=0, there were two subjects only that each had one variable missing) were also excluded.

Ethical approval

This study was approved by the Medical Ethic Committee of Guangzhou Women and Children's Medical Center. All the data already existed in the computerized medical recording system, so no informed consent was needed.

Data collection procedures

All data were collected in the database of medical records in the Guangzhou Women and Children's Medical Center during June to July 2012, by two trained attending physicians independently with the standard data extraction sheets [9]. All the NI cases were identified by the associate chief doctor or chief doctor and the experienced infection control staff. Two postgraduate medical students were responsible for the data entry to the computers separately and double checked the data to ensure correct entry.

Measurements

The measurement and the variables of demographic and family characteristics, the clinical, and the healthcare procedures in the study were categorized and defined as follows:

The measurements of NI in NICU

– Incidence rate of NI: the new cases of NIs occurred in a given time period (year)/all the persons at risk in that given time (year) *100
– Incidence density: the new episodes of NIs occurred in a given time period /the number of patients-days in a given time*1,000
– Ventilator-associated pneumonia infection rate: new episodes of ventilator-associated pneumonia in a given time/ the number of ventilator-days *1,000
– Central line-associated bloodstream infection rate: new episodes of central line-associated bloodstream infections / the number of central line-days*1,000

Demographic characteristics of the infants

– Sex: male as the reference group.
– Age: days as the units were recorded.
– Gestational age: The number of weeks that passed from the first day of the last menstrual period (LMP) to the birth day. It was categorized into two groups: early preterm birth: born before 32 weeks of pregnancy and non–early preterm birth born after 32 weeks of pregnancy which was the reference group.
– Birth weight: very-low–birth-weight (VLBW) ≤ 1,500 g and non-VLBW > 1,500 g (reference group).
– Congenital malformation: defined as the infants with congenital heart diseases, congenital chromosomal abnormalities, tumor and congenital abnormal body structure which required medical, surgical or cosmetic treatments. Infants without congenital malformation were the reference group.
– Singletons: categorized into two groups: singleton (reference group); twins or triplets.

The family characteristics

– Maternal age: the age of the mother at the time of delivery.
– Advanced maternal age: defined as the women older than 35 years when she gave birth to her first child.
– Pregnancy complications: defined as the symptoms and problems which were associated with the pregnancy for the present infants. Diagnosis was based on "The International Classification of Diseases, 10th Revision, Clinical Modification" (ICD-10-CM) [12]. These mainly included diabetes mellitus in pregnancy (024.901) and severe pre-eclampsia (014.101).

Healthcare procedures associated variables

All the variables related to healthcare procedures, such as receipt of pulmonary surfactant and umbilical vein catheterization (UVC), the peripherally inserted central catheters (PICC) or arterial cauterization (or both) and urinary catheter, intubation and ventilation, receipt of nasal continuous positive airway pressure (NCPAP), use of probiotics and antibiotics, and receipt of operation were counted from admission until the onset of NI occurring in case groups or until discharge of non-infection control group.

The duration of ventilation and the duration of using probiotics were recorded from the day of receipt of the procedures or probiotics until the procedures were completed or until the NI, whichever was earlier.

Data analysis

The authors described the NI rate, infection density and device-related infection rate, seasonal distribution, the type of infection, and the pathogens identified to reveal the profile of NI in NICU.

The authors compared the general characteristics, the family characteristics, and healthcare related procedure characteristics of cases when they had the first episodes of NI after admission with the control group using Chi square test for discrete variables and independent t-test for continuous variables to examine the association with NI. For patients with more than one episode of NI, the authors only considered the first episode in the case control analysis.

First, the authors applied each of the variables to fit the binary regression model, then they conducted a multivariate model with variables which showed a statistically significant association with NI in univariate analysis (p value less than 0.05) to predict the outcome.

As some variables in the multivariate model (adjusted OR) showed inconsistent results with univariate model (crude OR), hence the authors check the multicollinearity within the model by correlation matrix. At last, they applied the multivariate model with stepwise methods with all the variables in the previous model to get the independent variables to NI.

Results

Incidence rate of NI

During the study period, there were 1,653 infants admitted to NICU. The total number of episodes of NIs was 103. The calculation indicated that the NI rate was 6.2 episodes per 100 patients (95% CI, 6.0 - 6.4 episodes per 100 patients).

Incidence density

The total patient-days were 24,547, in which resulted 4.2 episodes per 1,000 patient-days (95% CI, 4.0-5.8 episodes per 1,000 patient-days) in the infection density.

Ventilation associated pneumonia infection rate

There were 24 episodes of ventilation-associated pneumonia during the study period, plus 7,069 MV days. The infection rate of ventilation-related pneumonia was 3.4 episodes per 1,000 MV days (95% CI, 3.2-3.5 cases per MV patient-days)

Central line-associated bloodstream infection rate

In 1,482 central line days, there were eight infected episodes; therefore the infection rate was 5.4 episodes per 1,000 central line days. (95% CI, 5.1 - 5.6 episodes per central line days)

Crude death rate

There were seven deaths in the NI cases and 75 deaths (including the death in the NI cases) among all patients admitted in NICU during the study period, resulting in a death rate of 8.6% and 4.5%, respectively.

There were 16 (20.0%) patients out of 81 infants had two NIs, while six (7.4%) out of 81 infants had three or more NIs. Among 103 episodes of NI in the study, pneumonia accounted for 34% of all the episodes (Table 1). The major agent of NI was Klebsiella pneumonia, which was most commonly cultivated in airway secretions, followed by Pseudomonas aeruginosa, which was more commonly cultivated in blood and airway secretions (Table 2).

Table 1. — *Distribution of 103 episodes of NI in NICU.*

Type of infection	First episodes (N=81)	Second episodes (N=16)	Third episodes or more (N=6)	Total (n=103)
Pneumonia	25(31%)	5(31%)	5(83%)	35(34%)
Sepsis	21(26%)	3(19%)	1(17%)	24(23%)
Enterocolitis	11(14%)	3(19%)	0	14(14%)
Meningitis	3(4%)	3(19%)	0	6(6%)
Thrush	7(9%)	1(6%)	0	7(7%)
Surgery wound infection	4(5%)	1(6%)	0	5(5%)
Other	12(15%)	0	0	12(12%)

Table 2. — *Distribution of 49 pathogens of NI in NICU No. (%) of pathogens identified.*

Genus, species	First episodes (N=40)	Second episodes (N=5)	Third episodes or more (N=4)	Total (n=49)
Klebsiella pneumonia	12(30%)	1(20%)	1(25%)	14(29%)
Pseudomonas aeruginosa	6(15%)	1(20%)	1(25%)	8(16%)
Candida albicans	5(13%)	0	0	5(10%)
Pathogenic escherichia coli	3(8%)	0	0	3(6%)
Enterococcus faecalis	3(8%)	0	0	3(6%)
Klebsiella oxytoca	2(5%)	0	0	2(4%)
Acinetobacter baumannii	2(5%)	0	0	2(4%)
Escherichia coli	1(3%)	0	1(25%)	2(4%)
Enterococcus faecium	2(5%)	0	0	2(4%)
Enterobacter cloacae	1(3%)	0	0	1(2%)
Candida glabrata	0	2(40%)	0	2(2%)
Others	4(10%)	1(20%)	1(25%)	6(4%)

Characteristics of study subjects related to NI (related to second outcome)

For 381 enrolled infants in the study, there were 35.8% females in the NI cases group and 36.3% females in the control group respectively; thus, there was no significant sex difference between groups. The mean age of NI cases group and control group were also similar. However, the number of early preterm birth in the NI cases group was 43.2%, which was much greater than the percentage in the control group (18%). Moreover, there was 9.9% VLBW infants in the NI cases group, which was much greater than in the control group (2.0%; $p < 0.001$). The proportion of infants with congenital malformation in NI cases group was 30%, which was much higher than the proportion in the control group (10%; $p < 0.001$). There were also significantly more twins/triples in NI cases group than in control group (35.8% *vs.* 17.6%; $p < 0.001$). Overall, there were significantly smaller gestational ages and birth weight, more congenital malformations, more twins or triplets in the NI cases group than in the control group (Table 3).

The mean age of mothers in two groups was similar, which was around 29 years. There were significantly more ad- vanced maternal age mothers in the NI cases group

Table 3. — *Background characteristics of infected infants and controls.*

Characteristics	Nosocomial infected cases (%) (N=81)	Non-infected controls (%) (N=300)	*p* value
Sex (female)	29(35.8%)	109(36.3%)	0.93
Age (days)	5.7±13.4	6.1±10.0	0.75
Gestational age			
Early preterm birth[a]	35(43.2%)	53(17.7%)	<0.001
Moderate and mild preterm birth[b]	28(34.6%)	121(40.5%)	
Term infants[c]	18(22.2%)	125(41.8%)	
Birth weight			
≤ 1,500g	31(38.3%)	47(15.7%)	<0.001
> 1,500g	50(61.7%)	253(84.3%)	
Congenital malformation	24(29.6%)	30(10%)	<0.001
Twins or triplets	29(35.8%)	53(17.6%)	

[a] Early preterm birth: gestational age ≤ 32 weeks
[b] Moderate and mild preterm birth: gestational age > 32 and < 37 weeks
[c] Term infants: gestational age ≥ 37 weeks

Table 4. — *Mother's characteristics of NI neonates and controls.*

Characteristics	Nosocomial infected cases (%) (N=81)	Non-infected controls (%) (N=300)	*p* value
Mean maternal age (yrs)	29.6±5.1	29.0±4.8	0.25
Advanced maternal age (yrs)	22(21.4%)	39(13.0%)	0.041
Older than 40 years	2(1.9%)	6(1.9%)	1
Pregnancy complications	53(17.7%)	22(21.4%)	0.46
Diabetes	6(5.9%)	16(5.3%)	0.77
Severe pre-eclampsia	6(4.9%)	8(2.7%)	0.47

Table 5. — *Healthcare procedures associated characteristics.*

Characteristics	Nosocomial infected cases (%) (N=81)	Non-infected controls (%) (N=300)	*p* value
Receipt of pulmonary surfactant	28(34.6%)	40(13.3%)	<0.001
Receipt of UVC[a]	35(43.2%)	39(13%)	<0.001
Duration of UVC (days)	3.6±4.5	1.1±3.0	<0.001
Receipt of PICC[b] / arterial catheter	28(34.6%)	34(11.3%)	<0.001
Duration of PICC / arterial catheter	5.4±11.8	2.5±9.7	0.044
Receipt of urinary catheter	20(24.7%)	46(15.3%)	0.013
Receipt of gastric tube	55(67.9%)	99(33.0%)	<0.001
Receipt of Intubation			
1 times	29(50.0%)	77(76.2%)	0.001
2 times	18(31.0%)	20(19.8%)	
3 times	4(6.9%)	3(3.0%)	
4 times or more	7(12.1%)	1(1.0%)	
Receipt of ventilation	52(64.2%)	103(34.3%)	<0.001
Duration of ventilations (days)	4.0±5.9	1.6±4.1	0.001
Receipt of NCPAP	21(25.9%)	41(13.7%)	0.011
Prophylactic antibiotics	73(90.1%)	212(70.2%)	<0.001
Duration of using prophylactic antibiotics	8.7±6.9	4.5±4.8	<0.001
Use of probiotics	19(23.5%)	108(36.0%)	0.034
Duration of using probiotics	2.3±5.4	3.7±6.8	0.05
Use of parenteral nutrition	62(76.6%)	205(68.3%)	0.17
Duration of using parenteral nutrition	8.6±9.0	6.7±8.6	0.08
Receipt of operation	29(35.8%)	36(12%)	<0.001
Duration of hospitalization (days)	53.1±69.78	16.6±16.1	<0.001

[a] Umbilical vein catheterization (UVC);
[b] Peripherally inserted central catheters (PICC).

than in the control group. Only 2% of the mothers were older than 40 years in both groups. The main complications of pregnancy in the mothers were diabetes mellitus and hypertension, which were approximately 6% and 5%, respectively, with no significant differences in both groups (Table 4).

Mean length of NICU stay was 53.1 days in NI cases group, which was much longer than those in non-infected control group (16.6 days); 92% of the infants' NICU stay in NI cases group were lasted for six to 109 days, and only two infants' NICU stay were far from the mean. After excluding two outliers (more than 454 days), the difference of NICU stay length was still significant ($p < 0.001$). There were more infants in NI cases group requiring exogenous pulmonary surfactants, UVC, PICC and urinary catheter (UC), intubation, ventilation, operations, which indicated that the NI cases group had more severe medical conditions than the non-infected control group (Table 5).

Due to the more medical procedures that were performed in NI cases group, the percentage of patients who had intravenous prophylactic antibiotics was greater. Furthermore, the authors found that more infants in the non-infected control group were given probiotics, and had longer duration than the infected case group.

Because of the incomplete data of the entire 12 months in 2009 and 2012, the authors compared all the episodes of four seasons in 2010 and 2011 (51 episodes in 2010 and 47 episodes in 2011). The finding is that there was no difference in the seasonal pattern of NI (Table 6). A better comparison should be based on incidence or incidence density, as the number of patients might vary in different periods. It is unfortunately that data were not available; therefore the authors could only compare the number of episodes.

Potential factors related to NIs

In order to predict whether or not the NI would occur, the authors applied the binary logistic regression analysis to background factors, which related to the NIs. Gestational age older than 32 weeks and females showed pro-

Table 6. — *NI episodes in four seasons of 2010 and 2011.*

Seasons	2010	2011	*p* value
Spring (Mar - May)	14(27.5%)	10(21.3%)	0.16
Summer (Jun - Aug)	14(27.5%)	6(12.8%)	
Autumn (Sep - Nov)	17(33.3%)	21(44.7%)	
Winter (Dec - Feb)	6(11.8%)	10(21.3%)	

Table 7. — *Crude and adjusted OR for background characteristics.*

Variables	Crude OR (95% CI)	*p* value (2-tailed)	Adjusted OR (95% CI)	*p* value (2-tailed)
Sex (female)	0.98 (0.59-1.63)	0.93	0.65 (0.31-1.34)	0.24
Birth weight < 2,000 g	2.84 (1.70-4.74)	<0.001	1.63 (0.61-4.30)	0.33
Gestational age > 32 weeks	0.28 (0.17-0.48)	<0.001	0.53 (0.21-1.38)	0.2
Congenital malformations	3.79 (2.06-6.96)	<0.001	4.37 (1.53-12.50)	0.006
Twins or triplets	2.6 (1.51-4.47)	0.001	3.88 (1.69-8.92)	0.001

*OR adjusted by sex, birth weight <2000g, gestational age >32 w, malformations, twins, intubation, vein or arterial catheter, ventilation, duration of ventilation, prophylactic antibiotics, duration of antibiotics, duration of probiotics, operation, parenteral nutrition, duration of parenteral nutrition, gastric tube feeding.

Table 8. — *Crude and adjusted OR for healthcare procedures.*

Variables	Crude OR (95% CI)	*p* value (2-tailed)	Adjusted OR (95% CI)	*p* value (2-tailed)
Receipt of intubation	4.97 (2.90-8.52)	<0.001	2.51 (0.92-6.81)	0.07
Receipt of vein or arterial catheter	5.77 (3.41-9.76)	<0.001	3.92 (1.76-8.70)	0.001
Receipt of ventilation	3.43 (2.05-5.73)	<0.001	0.52 (0.18-1.49)	0.22
Ventilation duration	1.1 (1.05-1.16)	0.071	1.02 (0.95-1.10)	0.62
Using prophylactic antibiotics	3.79 (1.75-8.19)	<0.001	1.17 (0.38-3.64)	0.78
Duration of using antibiotic	1.14 (1.09-1.19)	<0.001	1.13 (1.05-1.21)	0.001
Using probiotics	0.55 (0.31-0.96)	0.04	2.95 (0.92-9.48)	0.07
Duration of probiotics	0.96 (0.92-1.01)	0.09	0.88 (0.80-0.96)	0.004
Receipt of operation	4.09 (2.31-7.25)	<0.001	6.17 (2.47-15.41)	<0.001
Receipt of parenteral nutrition	1.51 (0.86-2.67)	0.15	0.98 (0.38-2.52)	0.97
Duration of parenteral nutrition	1.02 (0.997-1.06)	0.08	0.94 (0.90-0.99)	0.02
Receipt of gastric tube feeding	4.3 (2.54-7.26)	<0.001	3.27 (1.51-7.09)	0.003

*OR adjusted by sex, birth weight <2000g, gestational age >32 w, malformations, twins, intubation, vein or arterial catheter, ventilation, duration of ventilation, prophylactic antibiotic, duration of antibiotics, duration of probiotics, operation, parenteral nutrition, duration of parenteral nutrition, gastric tube feeding.

Table 9. — *Correlation matrix*

	Constant	Sex (female)	Birth weight < 2,000 g	Gestational age >32	weeks	Congenital malformation	Twins or singletons	Receipt of intubation	Receipt of vein or arterial	catheter	Ventilation	Duration of ventidur	Prophylactic antibiotic	Duration of prophylactic antibiotic	Using probiocs	Duration of using probiotics	Operation	Receipt of parenterna
Constant	1.000	-.073	-.625	-.684	-.106	-.301	-.056	.009	-.094	-.036	-.245	-.141	-.115	.127	-.053	.008	-.040	-.025
Sex (female)	-.073	1.000	-.074	-.186	.039	-.238	-.033	-.098	-.096	.152	-.012	.009	.026	.130	-.096	-.007	-.065	-.037
Birth weight < 2,000 g	-.625	-.074	1.000	.368	.282	-.101	-.121	-.246	.122	.031	-.029	.063	.127	-.086	.029	-.166	.067	-.093
Gestational age > 32 weeks	-.684	-.186	.368	1.000	-.186	-.019	.081	.135	.156	.028	-.051	.115	-.175	.090	-.070	-.147	.195	-.014
Congenital malformation	-.106	.039	.282	-.186	1.000	.060	-.134	.027	-.104	.110	-.034	-.034	.201	-.052	-.192	-.059	-.031	.092
Twins or singletons	-.301	-.238	-.101	-.019	.060	1.000	.174	.036	-.090	-.110	.182	.035	.118	-.294	.122	.027	-.006	-.040
Receipt of intubation	-.056	-.033	-.121	.081	-.134	.174	1.000	-.097	-.568	-.082	-.095	.103	-.069	-.018	.087	.029	-.042	-.135
Receipt of vein or arterial catheter	.009	-.098	-.246	.135	.027	.036	-.097	1.000	-.002	.014	-.073	.080	.007	-.038	.144	-.104	-.072	-.003
Ventilation	-.094	-.096	.122	.156	-.104	-.090	-.568	-.002	1.000	-.310	-.084	.061	.093	-.100	-.297	-.043	.089	-.022
Duration of ventilation	-.036	.152	.031	.028	.110	-.110	-.082	.014	-.310	1.000	-.032	.014	-.029	.165	.034	.062	-.212	-.001
Prophylactic antibiotic	-.245	-.012	-.029	-.051	-.034	.182	-.095	-.073	-.084	-.032	1.000	-.451	.053	-.048	.012	.025	-.049	.074
Duration of prophylactic antibiotic	-.141	.009	.063	.115	-.034	.035	.103	.080	.061	.014	-.451	1.000	.075	-.095	.030	.038	-.210	-.006
Using probiotics	-.115	.026	.127	-.175	.201	.118	-.069	.007	.093	-.029	.053	.075	1.000	-.743	.091	-.113	-.019	.097
Duration of using probiotics	.127	.130	-.086	.090	-.052	-.294	-.018	-.038	-.100	.165	-.048	-.095	-.743	1.000	-.087	.047	-.116	-.049
Operation	-.053	-.096	.029	-.070	-.192	.122	.087	.144	-.297	.034	.012	.030	.091	-.087	1.000	-.058	-.085	.210
Receipt of parenterna nutrition	.008	-.007	-.166	-.147	-.059	.027	.029	-.104	-.043	.062	.025	.038	-.113	.047	-.058	1.000	-.408	-.180
Duration of parenteral nutrition	-.040	-.065	.067	.195	-.031	-.006	-.042	-.072	.089	-.212	-.049	-.210	-.019	-.116	-.085	-.408	1.000	-.155
Receipt of gastric tube	-.025	-.037	-.093	-.014	.092	-.040	-.135	-.003	-.022	-.001	.074	-.006	.097	-.049	.210	-.180	-.155	1.000

tection to NI. Gestational age older than 32 weeks group was 72% less likely to get NI [OR 0.28 and $p < 0.001$]. However, after adjusted by all the significant background factors in the univariate regression model and all healthcare procedure related factors, the association was not significant. Females were 2% less likely to get NI than males, but it was not striking [OR 0.98, $p = 0.24$]. Infant with congenital malformation had significant harmful effect on the NI in both univariate and multivariate models. Congenital malformation infant was about four times more likely to get NI than the infant who was free from the disease. Birth weight less than 2,000 g and multiple births also showed harmful to NI. Only the multiple births founded significant association after adjustment (Table 7).

Compared to the infants without operations, those infants who underwent surgery were five times more likely to get NI. The harmful effects were showed in both univariate model and multivariate model. The infants with gastric tubes and received vein or arterial catheters also were significantly more likely to get NI than the controls after adjusted by all the other factors [OR 3.27, $p = 0.003$] (Table 8).

Duration of using probiotics showed protection to NI. Infants who used probiotics longer were less likely to get NI. The association was significant after adjustment [OR 0.88, $p = 0.004$]. Moreover, for the infants who received intubation, the length of ventilation and the use of prophylactic antibiotics showed to be harmful to NI, however after adjusted the effects were not significant.

Nevertheless, some factors such as parenteral nutrition and ventilation were harmful to NI in the univariate model, then showed protection in multivariate model. Just like those who had probiotics, the factor evened and then went to the opposite direction after adjustment.

As some factors (e.g. using probiotics and receipt of ventilation) showed inconsistent results with the univariate model, the authors investigated the multi-collinearity within the model by correlation table (Table 9). The score of using probiotics and duration of using probiotics was -0.743, meanwhile the score of intubation and ventilation was - 0.568. Due to the absolute score of some factors in the correlation matrix that was greater than 0.7, the authors applied the forward conditional methods of the regression model.

Independent factor related to NIs

Receipt of operation [OR 5.32, $p < 0.001$], with vein or arterial catheter [OR 4.76, $p < 0.001$] and gastric tube feeding [OR 3.88, $p < 0.001$] and with congenital malformation [OR 4.43, $p = 0.002$], multiple births [OR 3.24, $p = 0.003$] had a higher risk to have NI. Gestational age >32 weeks [OR 0.35, $p = 0.01$], the length of using probiotics [OR 0.88, $p = 0.005$], and length of using parenteral nutrition [OR 0.94, $p = 0.008$] were the protection factors of NI (Table 10).

Table 10. — *Independent factors for NI.*

Variables	Adjusted OR	95% CI	*p* value (2-tailed)
Gestational age > 32 weeks	0.35	0.16-0.80	0.01
Congenital malformation	4.43	1.72-11.39	0.002
Twins or triplets	3.24	1.51-6.95	0.003
Duration of use prophylactic antibiotics	1.13	1.06-1.20	<0.001
Duration of parenteral nutrition	0.94	0.90-0.99	0.008
Receipt of vein or arterial catheter	4.76	2.27-9.96	<0.001
Receipt of operation	5.32	2.30-12.30	<0.001
Receipt of gastric tube feeding	3.88	1.87-8.05	<0.001
Duration of probiotics	0.88	0.81-0.96	0.005

Discussion

The essential measure to NI control is to implement close monitoring and to promote full awareness of the risk factors of infection. To accurately measure the extent of NI is the first step of controlling. There are many confounders which influence the infection rate, such as different medical conditions and the number of admissions. Effective confounder control, more accurate measurement, and timely identification of the patients at risk, as well as comparison with other institutes are needed. Therefore in recent years, more and more studies were done not only on incidence rate of infection, but also on incidence density and device-associated healthcare-associated infection rate (DA-HAI rate) to measure NI. In the present study, the authors used all of these ways to measure the extent of NI.

According to the present results, the NI rate of our NICU was 6.2 episodes per 100 patients (95% CI, 6.0 - 6.4 episodes per 100 patients). These were similar to the data from NICU of other cities in China which infection rate varied from 7% - 11.6% [7-9,13,14], and American countries and European countries which varied from 6% - 40% [4, 15-17]. The present incidence density was 4.2 episodes per 1,000 patient-days (95% CI, 3.4-5.0 episodes per 1,000 patient-days) which was lower than that from Asian countries such as Korea, in which the incidence density was on average 15.1 infections per 1,000 patient days [18]. The wide range of infection rate was due to the different kinds of patients in NICU, which could be the percentage of preterm infants and the infants who required surgery among all patients admitted, because preterm infants and post-surgical infants were more vulnerable. There were about 10% - 16% of the patients admitted for surgery and 50% - 60% were preterm births yearly in the present NICU, hence the infection rate was relatively low. In the meantime, the central line-associated bloodstream infection rate and the ventilation associated pneumonia infection rate were also lower than the data from International Infection Control Consortium (INICC), which were 5.4 episodes per 1,000 central line days and 3.4 episodes per 1,000 MV days versus 12.2 episodes per central line days and 9.0 episodes per 1,000 MV days, respectively. The probable reasons were the low utilization of central line and ventilation. The present central line utilization ratio (number of central line-days/ number of patient-days) [19] was about 11% and ventilation utilization ratio (number of ventilator-days / number of patient-days) [19] was about 10%, which were much lower than the data from INICC surveillance reports from 2003 to 2008, which were 25% and 13%, respectively [20]. Meanwhile, the present central line-associated bloodstream infection rate and the ventilation associated pneumonia infection rate were also lower than the data from INICC, which were 5.4 episodes per 1,000 central line days and 3.4 episodes per 1,000 MV days versus 12.2 episodes per central line days and 9.0 episodes per 1,000 MV days.

In the present study, pneumonia was the most common type of infection, which accounted for 34% of all types of infections. The result was consistent with a study from Beijing and a study from Guangzhou [7, 21]. However, many studies from other countries found that sepsis was the most common type of infections [15, 22, 23]. One of the reasons of the high incidence rate of pneumonia may be that its diagnosis was easy to confirm by the doctor. Pneumonia can be diagnosed by tachypnea of the infants and the characteristic manifestation of chest X ray, which were easily observed and available. However, it was relatively difficult to diagnose sepsis. Positive blood culture is still the golden standard of sepsis diagnosis. Prophylactic antibiotics are commonly used in neonates, especially preterm infants, and the small blood sample increases false negative rate of the blood culture, leading to missed diagnosis of sepsis. Some studies showed high incidence of urinary tract infection [20, 24], but this could not be confirmed by the present study. This could be due to the low utility ratio of urinary catheter.

The first three pathogens of NI in the present study were Klebsiella pneumonia, Pseudomonas aeruginosa, and Candida albicans, and these three pathogens represented 55% of all episodes of NIs. Some studies found that gram-positive cocci caused the largest proportion of infection, including Methicillin-resistant Staphylococcus aureus (MRSA), and vancomycin-resistant enterococci [25]. A Japanese NI study also showed that their pathogens were dominated by MRSA [26]. However the study from Washington University showed that the trend was changing over the past five years, and the dominant bacteria in NI was gram-negative bacillus [27]. The study was consistent with the present findings. The bacterial type varied in different areas. However, the common feature was the development of multi –resistant bacteria. Broad-spectrum antibiotics, use of dexamethasone, and prolonged hospitalization were the risk factors of infection [28,29]. The present study did not include the information of drug sensitive tests. The present authors need to further explore on this in the future. Nosocomial fungal infection is a great threat to infants, especially to the preterm infants with devastating consequences [30]. After regular prophylactic use of antifungal drugs to the VLBW infants with broad-spectrum antibiotics, the incident rate is decreasing [31].

Few studies reported seasonal changes of NI in NICU. The present study found no association between seasons and NI. The possible reason may be the setting of the present NICU is an isolation ward. Anyone that enters it must change their clothes and shoes, and wear a cap and mask. Visitors and parents are not allowed to go into the ward. Most of the patients were admitted at their first day of life. NICU is equipped with central air purification. Meanwhile, all the infants in NICU were under incubation, which provided a stable temperature and humidity environment. Hence there was less chance to be infected from outside of the ward. A study from the UK reported the seasonality of invasive Candida infection in preterm low birth weight infants. They collected in total 52 data over eight years and 73% of all cases developed infection during September to February [32]. Another study in NICU of Guangzhou, China showed nosocomial invasive fungal infection occurred in the Spring which was statistically significantly higher than that in other seasons (30). One possible reason of the seasonal trend may be that not all the NICU wards are isolated and the fungal conidia outside could have an influence on the inside fungal conidia level [33].

The present study showed that the birth weight and gestational age were inversely associated with NI. Many studies found the same association with birth weight and gestational age and the rate of NI. Lower birth weight and earlier gestational age increased the risk of NI [4,34,35]. The survey of Vermont-Oxfort neonatal network on 36 NICU in the USA found that the incidence of nosocomial sepsis in NICU was 26% in the infants with birth weight 501 g to 750 g; 22% in infants birth weighing from 751 g to 1,000 g; 15% in infants weighing 1,001 g to 1,250 g, and 8% in infants weighing from1,251 g to 1,500 g [36]. A report of INICC surveillance in 2002 showed the central line-associated bloodstream infection rate in birth weight less than 1,000 g preterm infants was 10.8 / 1,000 central line days, the ventilation associated pneumonia infection rate was 3.3 / 1,000 MV days; the central line-associated bloodstream infection rate in infants with a birth weight from 1,001 g to 1,500 g was 6.4 / 1,000 central line days, the ventilation associated pneumonia infection rate was 2.5 / 1,000 MV days; the central line-associated bloodstream infection rate in infants with birth weight from 1,501 g to 2,500 g was 4.1 / 1000 central line days, ventilation associated pneumonia infection rate was 2.1 / 1,000 MV days; the central line-associated bloodstream infection rate in preterm infants with birth weight heavier than 2,500 g was 3.7 / 1,000 central line days, ventilation associated pneumonia infection rate was 1.4 / 1,000 MV days. Low birth weight and early gestational age were indicators of the severity of disease. However, the research also showed, in multivariate analysis, that early gestational age was the independent risk factor, rather than birth weight [37] and the present study had the same conclusion.

Congenital malformation included digestive tract malformations, congenital heart diseases, and trauma in the present study, all of which accounted for circa 30% infants in the infected group, and in the non-infected control group it only accounted for 10% infants. The difference was significant. After adjusting for all of the other variables, congenital malformation had around a five-fold risk of NI as compared to the infants without. Congenital malformation contributed to the morbidity and mortality of neonates and infants. A study revealed that gastrointestinal malformations were associated with a mortality rate of 60.9% as compared with 21.1% in those with central nervous system malformations [38]. Digestive tract malformation accounted for around half of malformation in the present study and most of them required surgery. This may partly explain why congenital malformation infants had much higher risk to NI.

A study found perinatal mortality in twins that was much higher than singletons, which was five times more than singletons. The incidence of malformation in twins was three times as compared to former singletons [39]. Birth weight of multiple birth babies was smaller than the singletons. The need to share the nutrition in the uterus, makes them weaker than singletons. The present result showed that twins or triplets had a three-fold risk of NI compared to singletons, even after adjusting for birth weight and gestational age.

In order to provide nutrition and medicine to severely ill infants, UVC or PICC are often needed. However, intravascular interventions were associated with blood stream infection [37]. Central line catheter and the length of catheter were the main risks of NI for neonates, especially for VLBW infants [10, 40]. The present study results contributed more evidence. A study showed that more than five days' UVC increased the risk of sepsis at least 21 times [40]. Another study found that when duration of PICC in situ was more than nine days, the risk of PICC infection increased three times [41]. Many central line associated infections were caused by Staphylococcus or other pathogens colonized in the skin around catheter. The colonized pathogens could migrate into the blood stream and cause sepsis [10]. A study found that in infants with birth weight less than 1,000 g, because of bacterial colonization in the catheter, taking a blood sample from the central line was one of the risk factors of central line related infection. On the other hand, using heparin and catheter disinfection were protecting factors [42]. The present study result was consistent with this. The present infants with central line catheter had circa a four-fold risk to get infection compared to those without the catheter.

Some studies showed that parenteral nutrition was a risk factor of nosocomial sepsis in preterm infants [40,43]. However in the present study, parenteral nutrition showed minor protective effect on NI (OR=0.94, $p = 0.008$). Around 45% of patients only received lipid free parenteral nutrition in the present study. Some studies only focused on patients received lipid containing parenteral nutrition [43]. Research has shown that intralipid increased mycethemia and bacteremia caused by malassezia species and coagulase negative staphylococci [23]. So the possible reason may be due to the different components of the parenteral nutrition. On the other hand, parenteral nutrition provided the energy and protein to the infants who were severely ill or preterm as they had insufficient intakes. Sufficient nutritional support was important to improve perinatal and long-term outcomes [44]. According to the present study, parenteral nutrition showed some increased risk of NI in the univariate model and this may be due to the close correction of low birth weight and small gestational age. After adjusting for these factors in multivariate model, parenteral nutrition became protective.

The present authors observed that receipt of gastric tube was an independent risk factor of NI in NICU (OR 3.88, $p < 0.001$), which concurred with previous studies [45]. Using gastric tube might directly damage the gastric mucosa, which was a barrier against infection [46]. Continuous tube feedings also weaken the acid barrier function of the stomach by increasing gastric acidity levels [9]. Repeating insertion was very common in NICU, due to difficulty of fixing the orogastric tube. Research found that there were some conditional pathogenic bacteria, such as staphylococcus epidermidis colonized in the nasopharynx and skin of 78% of infants after two weeks' admission. Transmission of the bacteria in NICU also occurred by the hands of healthcare staff. A study showed a significant relationship between acquisition of bacteria by patients and oropharyngeal and healthcare instrumentation, including the use of suctioning, and use of gastric feeding tubes [46].

Bacterial colonization is a precursor to NI. The bacterial colonization rate indicates the risk of occurrence of bacterial the infection of the bacterial in NICU. Severe diseases of the newborn and the widespread use of antibiotics in NICU result in conditional pathogenic bacteria colonization in the body. Lactic acid bacteria and bifidobacteria are the normal colonization bacteria, which inhibit the growth of aerobic and facultative anaerobic bacteria, and ultimately achieve the balance of the intestinal flora. There was evidence showing the benefit of enteral probiotics to severe necrotizing enterocolitis (NEC) in VLBW infants [47]. A meta-analysis studied on the prophylactic use of enteral probiotics in prevent antibiotic-associated diarrhea showed the prophylactic use of probiotics reduced the risk of antibiotic-associated diarrhea in neonates (OR=0.28, 95% CI=0.20-0.38). The present study also found that the duration of using prophylactic enteral probiotics was associated with a reduced risk of NI (OR= 0.88, $p = 0.005$). The longer duration of using prophylactic enteral probiotics, the infants in NICU were less likely to develop NI. Future studies should also focus on the duration of use to guide clinical applications.

According to the present study, the proportion of prophylactic use of antibiotic in NI infants (90%) were more than that of non-infected infants (70%), and the mean length of using antibiotics (8.7 ± 6.9 days) in infected infants was longer than non-infected infants (4.5 ± 4.8 days), and the difference was significant. After adjusting for the background factors and healthcare procedures related factors, prophylactic use of antibiotics showed a slightly harmful effect of NI (OR 1.13, $p < 0.001$). The prevalence of prophylactic of antibiotics use was relatively high in the present NICU compared to a general hospital in US, the use of antibiotics in premature infants was much higher, which was 60% in suburban hospital and 43% in the inner city hospital [48]. Such comparison may not be so fair, because of the different composition of patients and healthcare policy. The present hospital included infants requiring surgery and the proportion of preterm infants was relatively high. Although there was biological justification to apply prophylactic antibiotics to preterm infants and infants with invasive intervention, there was no evidence that it could improve the overall mortality of preterm infants at present [49]. A study found that prior use of third-generation cephalosporin was a risk factor for ESBL-producing E. coli or K. pneumonia infection [50]. The present study showed the duration of use prophylactic antibiotics had some harm effect on NI. More researches are needed to evaluate the duration and timing of prophylactic antibiotic use.

The present authors observed that the infants undergoing surgery were five times as likely to get NI in NICU compared to the infants who did not need it. Surgery destroys the integrity of the skin, and foreign bodies, such as drainage, can let pathogens invade into the body more easily. Because of the defects in inherent immunity, and neutrophil activation, preterm infants were more susceptible than older infants. Infants after surgery may have decreased feeding, which worse the situation. The infants who required surgery were often in serious condition and needed to spend more time in hospital. Hence they had more chance to have invasive intervention. The site of surgery, the duration, and the environment of the operating room had an influence on the risk of infection. A retrospective study showed that skin preparation, urinary catheter, and procedure duration were risk factors of surgical site infection (SSI) [51].

Conclusion

NI rate of NICU has been increasing in the past decade, which is related to higher morbidity and mortality, prolonged hospital stay and increased hospitalization expenses [3]. A multicenter prospective cohort study found that the mortality of neonates with NI was five-fold as neonates without infection [22]. As the present study showed that the NIs in NICU are associated not only with intrinsic factors but also to factors related to the healthcare procedures. Hence, to reduce the NI rate depends on enhancing the healthcare level.

The present study is one of the few studies comprehensively assessing the risk factors associated with NIs of NICU in China. The findings of the study could provide a better understanding in the epidemiology of NI in NICU in China, and the relevant evidence for prevention strategies.

The present study identified the infection rate of NI in a NICU of Guangzhou, China. Gestational age < 32 weeks, congenital malformation, twins or triplets, gastric tube feeding, surgical operation, duration of prophylaxis antibiotic use, duration of probiotic use, and duration of parenteral nutrition were the risk or protective factors associated with NI in NICU. Multicenter prospective study may be conducted in the future to investigate specific risk factors on different birth weight or gestational age of China. The present study also provided information for the prevention strategies of NIs and may improve the healthcare service level.

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