Clinical features and antimicrobial susceptibility profiles of culture-proven neonatal sepsis in a tertiary children’s hospital, 2013 to 2017

Xiaoxia Li, MS\textsuperscript{a}, Xiangyu Ding, MS\textsuperscript{a}, Peng Shi, MS\textsuperscript{b}, Yiqing Zhu, MS\textsuperscript{a}, Yidie Huang, MS\textsuperscript{a}, Qin Li, MS\textsuperscript{a}, Jinmiao Lu, MS\textsuperscript{a}, Zhiping Li, PhD\textsuperscript{a,*}, Lin Zhu

Abstract
Neonatal sepsis (NS) remains a major cause of morbidity and mortality in neonates, but data on the etiology and antibiotic susceptibility patterns of pathogens are limited. The aim of this study was to analyze the clinical characteristics, risk factors, and the antibiotic susceptibility patterns of pathogenic microbes associated with NS at a tertiary children’s hospital in Shanghai, China.

Episodes of blood culture-proven sepsis in the neonatal intensive care unit (NICU) of Children’s Hospital of Fudan University from January 2013 to August 2017 were retrospectively reviewed. Collected data included demographics, perinatal risk factors, clinical symptoms, laboratory values, microbiology results and their antimicrobial susceptibility. Data for early-onset neonatal sepsis (EONS) and late-onset neonatal sepsis (LONS) were compared.

The 341 of 976 culture-positive cases were selected, including 161 EONS cases (47.21% of 341) and 180 LONS cases (52.79% of 341). 635 incomplete cases were excluded. There was significant difference in risk factors between the EONS group and LONS group including birth weight, gestational age, 1-minute Apgar score, respiratory support, and the use of peripherally insertion central catheter (PICC). Clinical symptoms such as fever, feeding intolerance, abdominal distension, and neonatal jaundice, and laboratory results such as hemoglobin and lymphocyte counts also showed between-group differences. Staphylococcus epidermidis (22.87%), Escherichia coli (9.68%), Alcaligenes xylosoxidans (9.38%) and Klebsiella pneumoniae (9.09%) remain the principal organisms responsible for neonatal sepsis. Most isolates of Gram-positive bacteria were sensitive to vancomycin, linezolid, minocycline and tigecycline, of which more than 90% were resistant to penicillin. Most isolates of Gram-negative bacteria were sensitive to amikacin and imipenem and resistant to amoxicillin. Fungus was sensitive to antifungal agents. Better medical decisions, especially early detection and appropriate initial antimicrobial therapy can be made after understanding the different clinical features and pathogens of EONS and LONS.

Abbreviations: CONS = coagulase-negative staphylococcus, CRP = C-reactive protein, EONS = early-onset sepsis, ESBL = extended-spectrum beta-lactamase producer, GBS = group B streptococcus, namely S. agalactiae, HGB = hemoglobin, LONS = late-onset sepsis, MRSA = methicillin-resistant Staphylococcus aureus, MSSA = methicillin-sensitive Staphylococcus aureus, NICU = neonatal intensive care unit, NS = neonatal sepsis, NT = not tested, PICC = peripherally inserted central catheter, PLT = platelet count, SMZ-TMP = Sulfamethoxazole-Trimeprprim, UAC = umbilical artery catheter, UVC = umbilical venous catheter, WBC = white blood cell count.

Keywords: drug resistance, early-onset sepsis, late-onset sepsis, neonatal sepsis, neonate, pathogenic

1. Introduction
Neonatal sepsis (NS) is used to describe a systemic condition of bacterial, viral, or fungal (yeast) infection that is associated with hemodynamic changes and other clinical manifestations which results in substantial morbidity and mortality.\textsuperscript{[1]} A consensus of the definition of neonatal sepsis has remained challenging.\textsuperscript{[2]} Neonatal sepsis can be classified into 2 subtypes, early-onset (EONS) and late-onset (LONS), depending upon whether the onset of symptoms is before 72 hours of life or later. EONS is defined as the start of sepsis symptoms within 72 hours of birth, and is caused by microorganisms present in the maternal genital tract before or at the time of birth.\textsuperscript{[2,3]} LONS, occurring after 72 hours from birth, is possibly due to bacteria transmitted from the hospital or the community during delivery.\textsuperscript{[4]}

Early diagnosis and treatment of the neonate with suspected sepsis are essential to prevent severe and life-threatening complications. Diagnosis of neonatal sepsis is a challenge due to variable and non-specific clinical symptoms and the difficulty of evaluating infection markers in the early stage.\textsuperscript{[5]} In clinical practice, treatment is complicated by the lack of sensitivity of bacterial cultures and the lack of accurate diagnostic markers. Antibiotic treatment is also increasingly complicated by the emergence of bacterial resistance, which has become a real challenge in several nurseries across North America.\textsuperscript{[6]}

China has
a vast territorial area and the prevalence and distribution of pathogens varies widely around the country. It is important to recognize the common pathogens and related drug susceptibility for individual hospital. In addition, it is crucial to dynamically monitor the local epidemiology of neonatal sepsis to detect any changes in infection patterns and drug susceptibility.\cite{7}

The objective of this article was to investigate the bacterial pathogens, to analyze the associated risk factors, and to confirm the antibiotic susceptibility pattern of common causative pathogens of neonatal sepsis in the Children’s Hospital of Fudan University, which may provide guidance on empirical antimicrobial treatment for neonatal sepsis.

2. Methods

2.1. Study design and patient population

This retrospective cohort study was conducted between January 2013 and August 2017, which was approved by the Ethics Committee of Children’s Hospital of Fudan University. Neonatal sepsis was defined as the growth of single potentially pathogenic organism (bacterium or fungus) from blood or cerebrospinal fluid (CSF) in patients with clinical and laboratory findings consistent with infection.\cite{8} Inclusive criteria: neonates (0–28 days) present with the risk factors and clinical symptoms of sepsis at the time of admission or who developed sepsis during hospitalization were included in this study. Exclusion criteria: among 976 culture-positive neonates, 635 cases with incomplete data were excluded.

The 341 selected cases were later divided into 2 subgroups: early-onset neonatal sepsis (EONS, onset of symptoms before 72 hours of life) and late-onset neonatal sepsis (LONS, onset of symptoms beyond 72 hours after birth and before 28 days).\cite{2,9} Patient medical records including clinical symptoms, hematological parameters, pathogenic features, and antimicrobial susceptibility were reviewed. Each patient’s data was recorded on a standardized data collection form. Blood cultures were obtained from infants with risk factors and clinical symptoms suggestive of sepsis. All blood samples were collected prior to initiation of antimicrobial therapy. To determine whether the organism is a real pathogen or a contaminant, a repeat blood culture was required. If the patient had 2 consecutive positive blood cultures, the neonatal sepsis was diagnosed. A few infants had more than one episode of sepsis. If the blood culture was still positive after 10-day appropriate antimicrobial treatment or a different organism was identified from a subsequent culture, it was considered an additional episode.\cite{7}

2.2. Susceptibility testing

The antimicrobial susceptibility for isolated pathogens was determined. Antimicrobial susceptibility testing of isolated pathogens was done with ATB susceptibility system (BioMérieux La Balmes-les Grottes, France) by the Kirby Bauer disk diffusion method according to Clinical and Laboratory Standards Institution (CLSI) recommendations.\cite{10}

2.3. Statistical analyses

Data was entered in Excel 2010 (Microsoft) and analyzed using SPSS 19.0 for Windows (IBM, Armonk). Distribution difference of categorical variables were assessed using Mantel-Haenzel chi-square test or Fisher exact test according to the need of the problem. To compare the Apgar score between 2 groups, independent samples t test was used. Two-side P values of < .05 were considered statistically significant.

2.4. Ethics approval and consent to participate

This retrospective study was conducted with approval from the Ethics Committee of Children’s Hospital of Fudan University. Due to the retrospective nature of the study, informed consent was waived.

3. Results

3.1. Occurrence rates of NS and risk factors

This was a retrospective study of hospital records from January 1, 2013 to August 31, 2017. Among 26,296 neonates admitted to the NICU, 3,454 (13.14%) neonates were diagnosed with neonatal sepsis. Of 3,454 neonates with clinical neonatal sepsis 976 (28%) and 2,478 (72%) had positive and negative culture results, respectively. For further study, cases with incomplete data were excluded and 341 cases were selected, which included 161 EONS cases and 180 LONS cases. Among these culture-proven septic neonates, the proportion of full term delivery (≥37 weeks), normal birth weight (>2500 g), and high Apgar score was higher in LONS group than that in EONS group. The proportion of preterm birth (≤37 weeks), low birth weight (≤2500 g), premature rupture of membrane, polluted amniotic fluid, need of respiratory support, use of peripherally insertion central catheter (PICC), duration of hospitalization and antibiotic treatment was higher in EONS group than that in LONS group. Other parameters such as gender, mode of delivery and nuchal cord had no significant differences between 2 groups (Table 1).

3.2. Clinical symptoms and hematological parameters of neonatal sepsis

Respiratory distress (39.13%), neonatal jaundice (70.19%), hypoglycemia (12.42%), pulmonary hypertension (14.19%) and neonatal asphyxia (22.98%) were the most common clinical manifestations of EONS according to the results. In contrast, fever (40.56%), feeding intolerance (49.44%), and abnormal lymphocyte counts, C-reactive protein (CRP) and platelet counts were more common in LONS group, while abnormal neutrophil counts and hemoglobin concentrations were more common in EONS group, while abnormal lymphocyte counts, C-reactive protein (CRP) and platelet counts were more common among LONS group. These clinical symptoms could be helpful in making more accurate early diagnosis and reducing the burden of disease caused by misdiagnosis and delayed diagnosis. Other clinical parameters including hypotonia/poor activities, respiratory failure, white blood cell (WBC) counts, procalcitonin (PCT) showed no significant statistical difference between EONS and LONS group (Table 2).

3.3. Pathogen distribution

The primary pathogenic microorganism of NS was Gram-positive bacteria, which accounted for 48.33% and 65.98% of all infections in EONS and LONS group, respectively. *Coagulase-negative staphylococcus* (CONS) was the most common Gram-positive bacteria, accounting for 72.41% and 67.97% in EONS and LONS group, respectively. Gram-negative pathogens accounted for 36.67% and 29.90% of all infections in EONS and LONS group, respectively, among which *Escherichia coli* occurred most commonly in LONS group (44.83%) and *Alcaligenes xylosoxidans* occurred most commonly in EONS.
Table 1

Risk factors in patients with early-onset and late-onset neonatal sepsis, 2013 to 2017.

| Clinical parameters               | Total   | EONS     | LONS     | P       |
|-----------------------------------|---------|----------|----------|---------|
| Gender                            |         |          |          |         |
| Male                              | 193 (56.60) | 90 (55.90) | 103 (57.22) | .81     |
| Female                            | 148 (43.40) | 71 (44.10) | 77 (42.78) |         |
| Mode of delivery                  |         |          |          |         |
| Vaginal delivery                  | 165 (48.39) | 75 (46.60) | 90 (50.00) | .53     |
| Caesarean section                 | 176 (51.61) | 86 (53.40) | 90 (50.00) |         |
| Gestational age (weeks)           |         |          |          |         |
| Extreme preterm < 28              | 26 (7.62) | 19 (11.80) | 7 (3.90) | .00      |
| Very preterm 28 to 32             | 61 (17.89) | 50 (31.10) | 11 (6.10) |         |
| Moderate/late preterm 32 to 37     | 80 (23.46) | 49 (30.40) | 31 (17.20) |         |
| Full-term ≥37                     | 174 (51.03) | 43 (26.70) | 131 (72.80) |         |
| Birth weight (g)                  |         |          |          |         |
| >2500                             | 193 (56.60) | 59 (36.60) | 134 (74.40) | .00      |
| 1500 to 2500                      | 77 (22.58) | 46 (28.60) | 31 (17.20) |         |
| 1000 to 1500                      | 51 (14.96) | 39 (24.20) | 12 (6.70) |         |
| <1000                             | 20 (5.86) | 17 (10.60) | 3 (1.70) |         |
| Premature rupture of membrane     | 53 (15.54) | 32 (19.90) | 21 (11.70) | .04      |
| Polluted amniotic fluid           | 33 (9.38) | 22 (13.70) | 11 (6.10) | .02      |
| Umbilical cord around neck        | 23 (6.45) | 9 (5.63) | 14 (7.80) | .42      |
| 1-min Apgar score                 | 122 (35.78) | 98 (60.87) | 24 (13.33) | .00      |
| 5-min Apgar score                 | 53 (15.54) | 100 (62.11) | 34 (18.69) |         |
| Respiratory support               | 20.50 (13.00, 50.25) | 42.5 (17.00,70.00) | 16.00 (11.00,27.00) | .00      |
| Antimicrobial treatment lasted (d) | 17 (11, 30.75) | 23.00 (12.00,45.00) | 15.00 (10.00,22.00) | .00      |

EONS = early-onset neonatal sepsis, LONS = late-onset neonatal sepsis.

Table 2

Clinical symptoms and Hematological parameters accompanied diagnoses in patients with early-onset and late-onset neonatal sepsis, 2013 to 2017.

| Clinical parameters               | Total   | EONS (161) | LONS (180) | P       |
|-----------------------------------|---------|------------|------------|---------|
| Clinical symptoms                 |         |            |            |         |
| Hypotonia/poor activities         | 125 (36.66) | 65 (40.37) | 60 (33.33) | .18     |
| Fever                             | 89 (26.10) | 16 (9.94) | 73 (40.56) | .00      |
| Feeding intolerance               | 137 (40.18) | 48 (29.81) | 89 (49.44) | .00      |
| Respiratory distress              | 69 (20.23) | 63 (38.13) | 6 (3.33)  | .00      |
| Abdominal distension              | 53 (15.54) | 16 (9.94) | 37 (20.56) | .00      |
| Neonatal jaundice                 | 174 (51.03) | 113 (70.19) | 61 (33.89) | .00      |
| Hypoglycemia                      | 25 (7.33) | 20 (12.42) | 5 (2.78)  | .00      |
| Neonatal asphyxia                 | 42 (12.32) | 37 (22.98) | 5 (2.78)  | .00      |
| Pulmonary hypertension            | 29 (8.50) | 24 (14.19) | 5 (2.78)  | .00      |
| Respiratory failure               | 29 (8.50) | 15 (9.32) | 14 (7.78) | .61      |
| Hematological parameters          |         |            |            |         |
| White blood cell counts (<8 or >12) \*10^3/L | 241 (70.67) | 121 (75.16) | 120 (66.67) | .09     |
| Lymphocyte counts (<0.8 or >4) \*10^9/L | 120 (35.19) | 39 (24.22) | 81 (45.00) | .00     |
| C-reactive protein (≥8) \*mg/L    | 88 (25.81) | 24 (14.91) | 64 (35.56) | .00     |
| Neutrophil counts (<0.8 or >4) \*10^9/L | 196 (57.48) | 105 (65.22) | 91 (49.56) | .00     |
| Platelet counts (<100 or >300) \*10^9/L | 113 (33.14) | 28 (17.39) | 85 (47.22) | .00     |
| Hemoglobin (<110 or >160) \*g/L   | 206 (60.41) | 115 (71.43) | 91 (50.56) | .00     |
| Procalcitonin (≥0.05) \*mg/mL     | 337 (98.83) | 159 (98.76) | 178 (98.89) | .99     |

The neonate could have more than one of the above clinical findings. EONS = early-onset neonatal sepsis, LONS = late-onset neonatal sepsis.

P < .05 means a significant difference between EONS and LONS groups.
group (40.91%). Fungal pathogens (3.5%) were relatively rare compared to bacteria, though they caused 27 (15%) of all LONS cases and 8 (4.12%) of all LONS cases. Among LONS group, Staphylococcus epidermidis followed by A. xylosoxidans were the most common organisms, causing 28 (17.39%) and 26 (14.44%) of all LONS cases, respectively. Among EONS group, S. agalactiae was the most common organisms, causing 28 (17.39%) and 26 (14.44%) of all EONS cases, respectively. Amid Staphylococcus aureus, Methicillin-resistant S. aureus (MRSA) was found in all EONS cases, while Methicillin-susceptible staphylococcus aureus (MSSA) was found in 83.33% of LONS cases. Klebsiella spp. of EONS (92.86%) and LONS (94.12%) were suspect of ESBL-production as they displayed resistance to cefotaxime or/and ceftazidime on disc diffusion testing (Table 3 and Table 5).

### 3.4. Antimicrobial Susceptibility

A high-degree resistance to common first and second line antimicrobials was observed for the main causative pathogens of NS. The susceptibility rate to the following drugs was high among Gram-positive bacteria: tigecycline, linezolid, and vancomycin (Table 4). None of the 32 strains of A. xylosoxidans was resistant to tigecycline (100.00%), levofloxacin (100.00%), meropenem (100.00%), and ceferazone/sulbactam (100.00%). None of the 33 strains of E. coli were resistant to ertapenem (100.00%), cefmetazole (100.00%), meropenem (100.00%), and amoxicillin/clavulanic acid (100.00%). The susceptibility rate to following drugs was high among Klebsiella spp.: amikacin (100.00%), levofloxacin (100.00%), fosfomycin (95.65%), and gentamicin (80.65%) (Table 5). Furthermore, all candida spp are sensitive to antifungal agents (Table 6).

### 4. Discussion

The diagnosis of NS is difficult because clinical symptoms, particularly in the early stage, are hard to differentiate from other neonatal diseases. Blood and cerebrospinal fluid (CSF) culture has been considered as the gold standard for detecting bacterial sepsis. However, body fluid culture is time-consuming and pathogens of NS are widely distributed. Besides, the overall culture positive rate was 28.26% (976/3454) in our study, which was lower than that in other reports. Therefore, for the early diagnosis and treatment, it is meaningful to continue to evaluate risk factors, clinical symptoms, accompanied diagnoses, pathogenic bacteria and antimicrobial susceptibility of neonatal sepsis.

By investigating and analyzing the risk factors in the perinatal period, we can better differentiate the diagnosis of EONS and LONS. Preterm birth, low birth weight, premature rupture of membrane, and amniotic fluid-contaminated neonates were more common among proven EONS cases, which resulted in higher rate of respiratory support and PICC, and longer duration of hospitalization and antibiotic treatment. In comparison, most

---

**Table 3**

| Pathogenic microorganisms | Total (n=150) | EONS (n=50) | LONS (n=100) |
|---------------------------|--------------|-------------|--------------|
| Gram-positive pathogens   |              |             |              |
| Staphylococcus aureus     | 128 (65.98)  | 62 (126.00)| 66 (65.98)   |
| Methicillin-resistant S. aureus (MRSA) | 31.76 (47/148) | NT | 62.50 (10/16) |
| Fungi                     |              |             |              |
| Candida guilliermondii    | 32 (26.61)   | 7 (10.61)   | 25 (26.61)   |
| Candida pelliculosa       | 10 (28.57)   | 8 (29.63)   | 2 (25.00)    |
| Other fungi               |              |             |              |

**Table 4**

| Antibiotics            | CoNS         | GBS           | S. aureus |
|------------------------|--------------|---------------|-----------|
| Ampicillin/Sulbactam   | 31.76 (47/148)| NT            | 62.50 (10/16) |
| Benzylpenicilline      | 26.23 (16/61) | NT            | 50.00 (2/4)   |
| Cefazolin              | 31.08 (46/148)| NT            | 62.50 (10/16) |
| Cefotaxime             | NT           | 95.00 (19/20) | NT         |
| Ceftriaxime            | 28.79 (19/66) | NT            | 50.00 (2/4)   |
| Ciprofloxacin          | 51.11 (46/90) | NT            | 91.67 (11/12) |
| Fosfomycin             | 54.24 (32/59) | 30.00 (6/20)  | 50.00 (2/4)   |
| Gentamicin             | 71.33 (107/150)| NT           | 93.75 (15/16) |
| Levofloxacin           | 56.38 (84/149)| NT            | 99.17 (12/12) |
| Linezolid              | 99.29 (139/140)| NT           | 100.00 (19/19) |
| Minocycline            | 100.00 (66/66)| NT            | 100.00 (7/7)  |
| Moxifloxacin           | 58.24 (53/91) | 89.47 (17/19) | 100.00 (12/12) |
| Oxacillin              | 32.95 (29/88) | NT            | 66.67 (8/12)  |
| Penicillin             | 9.59 (14/146) | 100.00 (20/20)| 6.25 (1/16)   |
| Tobramycin             | 76.97 (98/126)| NT            | 84.62 (11/13) |
| Teicoplanin            | 86.00 (129/150)| NT           | 100.00 (16/16) |
| Ticarcillin            | 98.81 (83/84) | NT            | 100.00 (9/9)  |
| Ticarcillin            | 100.00 (100/100)| 100.00 (5/5)| 100.00 (15/15) |

GBS = group B streptococci, namely S. agalactiae, NT = not tested, SMZ-TMP = sulfamethoxazole-trimethoprim.
neonates with LONS were characterized by normal birth weight, full-term birth, and higher Apgar score. A study of neonates with EONS in the UK found that risk factors were present in 78% cases, but in almost half (17 of 35) of the cases, the only predictor was preterm labor. A further study classified LONS into community-acquired (neonates admitted from home) and hospital-acquired (neonates got infections in the NICU and blood culture was done before use of antibiotics) groups. There was no significant difference in clinical features between the 2 groups, but the hospital-acquired LONS neonates were more likely to be preterm. Compared with this report, most LONS cases in our study were community-acquired, among which risk factors were relatively rare. Gender, mode of delivery and nuchal cord showed no difference between 2 groups in our study, which was accordance with other reports.

Early initiation of antimicrobial therapy is frequently delayed because the first clinical symptoms of sepsis are non-specific. In our study, we found that clinical manifestations in order of frequency were neonatal jaundice, feeding intolerance, hypotonia/poor activities, fever, and respiratory distress. These findings were similar to other studies. The incidence of respiratory distress, neonatal jaundice, hypoglycemia, neonatal asphyxia and pulmonary hypertension was higher in EONS group than that in LONS group, while fever, feeding intolerance and abdominal distension were more common in LONS group. The clinical manifestations of EONS and LONS were different because of the causes and the timing of onset and the speed of development. The differences we observed were based on the assumption that early-onset infections were presumably transmitted perinatally from the mother and late-onset infections were acquired postnatally from an environmental source, which resulted in differences of symptom severity, pathogen distribution, and antibiotic susceptibility. Therefore, it is necessary to identify the early clinical manifestations and reduce the rate of underdiagnoses and misdiagnosis.

In clinical work, various laboratory data such as CRP, WBC counts, lymphocytes, neutrophils are often used to support the diagnosis of sepsis. We found that the values of PCT, WBC counts and hemoglobin are more likely to be abnormal compared with C-reactive protein and platelet counts. However, some studies considered CRP to be an indicator of both sensitivity and specificity. They regarded a CRP value >10 mg/L combined with a neutrophil ratio >0.25 as a criterion to start antibiotic therapy. In our study, the proportion of abnormal neutrophil

### Table 5
Selected antimicrobial susceptibility patterns from the main gram-negative bacteria of septic neonates, 2013 to 2017.

| Antibiotics | Alcaligenes xylosoxidans | Escherichia coli | Klebsiella spp. |
|-------------|-------------------------|-----------------|----------------|
| Amoxicillin  | 100.00 (5/5)            | 100.00 (1/1)    | 0 (0/3)        |
| Amikacin    | 84.38 (27/32)           | 100.00 (33/33)  | 100.00 (30/30) |
| Ampicillin  | 21.88 (7/32)            | 28.00 (7/25)    | 0 (0/15)       |
| Ampicillin/| 75.00 (21/32)           | 36.36 (12/33)   | 8 (2/25)       |
| Cefadroxil  | 0.00 (0/32)             | 50.00 (16/32)   | 6.45 (2/31)    |
| Ceftaxime   | 87.50 (28/32)           | 93.94 (31/33)   | 35.48 (11/31)  |
| Cefmetazole | NT                      | 100.00 (8/8)    | 25.00 (3/12)   |
| Cefoperazone/| 100.00 (32/32)         | 96.97 (32/33)   | 22.58 (7/31)   |
| Ceftaxime   | NT                      | 53.13 (17/32)   | 6.90 (2/20)    |
| Cefotetan   | 3.13 (1/32)             | 100.00 (23/23)  | 69.23 (9/13)   |
| Cefazidime  | NT                      | 81.82 (27/33)   | 12.90 (4/31)   |
| Ceftriaxone | 0.00 (0/32)             | 56.52 (13/23)   | 7.69 (1/13)    |
| Cefuroxime  | 0.00 (0/30)             | 53.13 (17/32)   | 6.67 (2/30)    |
| Ciprofloxacin| 71.88 (23/32)           | 48.48 (16/33)   | 77.42 (24/31)  |
| Ertapenem   | NT                      | 100.00 (26/28)  | 70 (14/20)     |
| Furadantin  | 0.00 (0/4)              | 88.89 (8/9)     | 25.00 (1/4)    |
| Gentamicin  | 3.13 (1/31)             | 69.70 (23/33)   | 80.65 (25/31)  |
| Imipenem    | 96.88 (31/32)           | 100.00 (33/33)  | 61.29 (19/31)  |
| Meropenem   | 100.00 (30/30)          | 100.00 (32/32)  | 64.52 (20/31)  |
| Levofloxacin| 100.00 (32/32)          | 47.83 (11/23)   | 100.00 (13/13) |
| Fosfomycin  | 0.00 (0/30)             | 96.55 (28/29)   | 95.65 (22/23)  |
| Piperacillin| 96.88 (31/32)           | 100.00 (33/33)  | 38.71 (12/31)  |
| SMZ-TMP     | 96.97 (32/33)           | 48.48 (16/33)   | 56.67 (17/30)  |
| Tigecycline | 100.00 (16/16)          | NT              | NT             |

NT = not tested, SMZ-TMP = sulfamethoxazole-trimethoprim.

### Table 6
Selected antimicrobial susceptibility patterns from the main fungi of septic neonates, 2013 to 2017.

| Antifungal agents | Candida guilliermondii n (%) | Candida pelliculosa n (%) | Candida albicans n (%) |
|-------------------|------------------------------|--------------------------|------------------------|
| Flucytosine       | 12 (100.00)                  | 10 (100.00)              | 7 (100.00)             |
| Amphotericin B    | 12 (100.00)                  | 10 (100.00)              | 7 (100.00)             |
| Fluconazole       | 12 (100.00)                  | 10 (100.00)              | 6 (85.71)              |
| Itraconazole      | 8 (66.67)                    | 7 (70.00)                | 6 (85.71)              |
| Vancyclohexol      | 12 (100.00)                  | 10 (100.00)              | 6 (85.71)              |
counts and hemoglobin was higher in EONS, while lymphocyte counts, CRP value, and platelet counts were more useful in identifying LONS. There is no consensus on hematological parameters, its results are influenced by the health status of the perinatal mother and her medications, age of onset and use of antibiotics of neonates. Recent study showed that since CRP may be elevated in neonates, CRP was not an accurate marker in picking up cases. Therefore, hematological parameters can only be used as an adjunctive tool for diagnosis, not such a “gold standard”.

Gram-positive infection was found to be more common than Gram-negative and fungal infection. CoNS was the major Gram-positive pathogen for both EONS and LONS, which was consistent with previous studies in Asia and other developing countries. However, true bacteremia caused by coagulase negative Staphylococcus is difficult to distinguish from blood culture contaminants. Another pathogen, GBS, is very common in foreign reports but very rare in China, which was consistent with other mainland China reports and may be related to the low rate of GBS colonization in Chinese pregnant women. With the development of perinatal medicine and neonatal first-aid technology, the survival rate of premature infants, especially very/extreme low birth weight (VLBW/ELBW) infants, has been increasing year by year. As the general condition of these newborns is often worse, invasive operation such as tracheal intubation and arteriovenous catheterization was often required. CoNS can produce biofilm and are likely to stick to medical instruments. CoNS infection is a major risk factor for premature infants. However, CoNS are normal flora of the human skin and mucosa whose pathogenicity has long been ignored and few systematic studies were left describing their epidemiology in human infections. Nevertheless, colonized CoNS pathogens have been reported to be responsible for human infections, particularly in immunocompromised hosts including neonates. The immune system of neonate is immature, and the skin and mucous membranes are too vulnerable to be an effective physical barrier. Relying on antibodies from mothers, newborns can fight against pathogenic microorganism. However, it is less effective for pathogens with low virulence. Besides, prolonged application of broad-spectrum antibiotics may disturb the normal flora of the body, relieves pathogens inhibited competitively, and lead to the proliferation of pathogenic bacteria. All these factors contribute to CoNS becoming the main pathogen of neonatal sepsis. The main Gram-negative bacteria in EONS was A. xylosoxidans (40.91%), followed by Klebsiella spp. (21.21%). A recent study reported A. xylosoxidans as one of the isolates. E. coli (44.83%) and Klebsiella spp. (29.32%) was the most common Gram-negative bacteria in LONS. These findings were similar to other studies.

Due to the potential false-negative results by various methods, empirical therapy for neonatal sepsis need to be initiated in suspected cases. An ideal choice of antimicrobial agents is to cover the most common pathogens without providing selection pressure for antibiotic resistance. Currently, the recommended first-line therapy includes gentamicin+flucloxacinil and gentamicin+amoxicillin/penicillin. This may be suitable in UK or other Western countries. However, in reports from many other countries or regions, different patterns of causative pathogens have been identified and the first-line therapy should be modified according to local epidemiology.

Although the resistance rate of some Gram-positive bacteria to gentamicin, rifampicin, levofloxacin and ciprofloxacin is low, these antibiotics may have severe side effects on liver, kidney, hearing and carilage development, which made them an inappropriate choice for newborns. Vancomycin, a glycopeptide antibiotic, is the most effective and economical drug for treatment of staphylococcal infections. However, vancomycin-resistant Staphylococcus has been reported, the rational use of vancomycin is of great significance in reducing and/or postponing the emergence of vancomycin resistant strains. A. xylosoxidans, Klebsiella spp., and E. coli showed low sensitivity to commonly used antibiotics while high sensitivity was observed for amikacin, imipenem, and meropenem. A similar trend of high resistance was reported by Ullah et al. Klebsiella spp. and E. coli showed high sensitivity to meropenem, imipenem, and fosfomycin, and can be used as a first-line treatment.

A recent study revealed that Candida spp. was responsible for 8 to 15% of hospital-acquired infections (HAIs). With the gradual improvement in the rate of diagnosis, prolonged duration of hospitalization, increasing use of invasive operation techniques, and the double infection caused by the nonstandard use of antibiotics are causing more fungal infections. Twelve cases of Candida guilliermondii (3.52%), 10 cases of Candida pelliculosa (2.93%), and 7 cases of Candida albicans (2.05%) were found among 341 positive specimens of blood culture. The risk for fungal sepsis is increased by colonization acquired vertically from maternal sources as well as horizontally from the NICU environment. The incidence rate of fungal infection in newborns was reported to be 10%. We should be careful and take certain prophylaxis to shorten the average length of hospitalization, standardize the use and dosage of antibiotics, avoid invasive operations, and improve the comprehensive diagnosis and treatment in the NICU to reduce the risk of infection. Apart from that, prophylactic administration of fluconazole during the first 6 weeks of life could reduce fungal colonization and invasive fungal infection in ELBW infants—those with birthweight <1000g.

Our study has several limitations. It was a descriptive study and therefore it was not possible to further analyze the association with potential risk factors. Due to the limitation of inclusion and exclusion criteria, the EONS group may include a few hospital-acquired LONS cases, resulting in the divergence of clinical parameters between the 2 groups. When analyzing hematology parameters, the percentage of neutrophils and lymphocytes are more meaningful than the absolute value, and the 95% confidence interval (CI) of these parameters should be adjusted based on the age.

5. Conclusion

In conclusion, S. epidermidis, E. coli, A. xylosoxidans, and K. pneumoniae remain to be the principal organisms responsible for neonatal sepsis in the tertiary children’s hospital. Better medical decisions on initial antimicrobial therapy, based on early detection, may be made after a constant updated understanding of the clinical features and pathogens of EONS and LONS.

Acknowledgments

We thanked selfless contributions of paediatricians and nurses in the Children’s Hospital of Fudan University, including pathogens and collection of specimens. We also thanked support of Department of Medical Statistics, Children’s Hospital of Fudan University that ensured the smooth implementation of this study.
Author contributions
All members of the writing committee contributed to the design and implementation of the study, analysis and interpretation of the data, and drafting of the report. The investigators had an opportunity to critically review results and contribute to the process of finalization of the report. The writing committee vouches for accuracy and integrity of the work, and accepts full responsibility for the content of the paper.

Conceptualization: Xiaoxia Li, Zhiping Li, Yiqing Zhu, Yidie Huang, Qin Li.
Data curation: Xiaoxia Li, Jinmiao Lu, Yidie Huang, Peng Shi.
Formal analysis: Yiqing Zhu, Yidie Huang.
Funding acquisition: Zhiping Li.
Investigation: Jinmiao Lu, Lin Zhu, Qin Li.
Methodology: Xiaoxia Li, Xiangyu Ding, Jinmiao Lu, Yidie Huang, Peng Shi.
Project administration: Qin Li.
Resources: Yiqing Zhu, Yidie Huang.
Software: Xiangyu Ding.
Supervision: Jinmiao Lu, Zhiping Li, Qin Li.
Validation: Yiqing Zhu.
Writing – original draft: Xiaoxia Li, Qin Li.
Writing – review & editing: Xiangyu Ding, Lin Zhu, Qin Li.

References
[1] Shane AL, Sánchez PJ, Stoll BJ. Neonatal sepsis. Lancet 2017;390: 1770–81.
[2] Wynn JL, Wong HR, Shanley TP, et al. Time for a neonatal-specific consensus definition for sepsis. Pediatr Crit Care Med 2014;15:523–8.
[3] Polin RA, Committee on F, Newborn. Management of neonates with suspected or proven early-onset bacterial sepsis. Pediatrics. 2012; 129:1006–15.
[4] T GE , Moges F, Eshete S, et al. Bacterial etiologic agents causing neonatal sepsis and associated risk factors in Gondar, Northwest Ethiopia. BMC Pediatr 2017;17:137–46.
[5] Hedegaard SS, Wisborg K, Hvas AM. Diagnostic utility of biomarkers for neonatal sepsis - a systematic review. Infect Dis (Lond) 2015;47: 117–24.
[6] Kan R, Razaghian HR, Larvoie PM. An immunological perspective on neonatal sepsis. Trends Mol Med 2016;22:290–302.
[7] Li Z, Xiao Z, Li Z, et al. 116 cases of neonatal early-onset or late-onset sepsis: A single center retrospective analysis on pathogenic bacteria. Zhonghua Er Ke Za Zhi 2003; 41:897–899.
[8] Yusuf D, Shalakht T, Awad S, et al. Clinical characteristics and epidemiology of sepsis in the neonatal intensive care unit in the era of multi-drug resistant organisms: a retrospective review. Pediatr Neonatol 2018;59:35–41.
[9] Afonso EDP, Blet S. Effect of gestational age on the epidemiology of late-onset sepsis in neonatal intensive care units - a review. Exp Rev Anti Infect Ther 2017;15:917–24.
[10] Shah BA, Padbury JP. Neonatal sepsis: an old problem with new insights. Virulence 2014;5:170–8.
[11] Collaboration IONIS. Characterisation and antimicrobial resistance of sepsis pathogens in neonates born in tertiary care centres in Delhi, India: a cohort study. Lancet Glob Health. 2016; 4:e752–60.
[12] Marín M, Valenzuela I, Vázquez A, et al. Prevention of early-onset neonatal group B streptococcal disease. Rev Obstet Gynecol 2013;6: 63–8.
[13] Saada D, Moxon ER. Handbook of Neonatal Infections: A Practical Guide. London: WB Saunders; 2003;151.
[14] Zhu M, Zheng G, Chen J, et al. Comparative analysis of the pathogens responsible for hospital acquired and community acquired late onset neonatal septicemia (Chinese). Chin J Pediatr 2008;46: 124–7.
[15] Hayun M, Alasiry E, Daud D, et al. The risk factors of early onset neonatal sepsis. Am J Clin Exp Med 2015;3:78–82.
[16] Shah GS, Budhathoki S, Das BK, et al. Risk factors in early neonatal sepsis. Kathmandu Univ Med J (KUMJ) 2006;4:187–91.
[17] Kadam P, Chuan H. Erratum to: rectocutaneous fistula with transmigration of the suture: a rare delayed complication of vault fixation with the sacropinous ligament. Int Urogynecol J 2016;27:505.
[18] Lu Q, Zhou M, Tu Y, et al. Pathogen and antimicrobial resistance profiles of culture-proven neonatal sepsis in Southwest China, 1990–2014. J Paediatr Child Health 2016;52:939–43.
[19] Baltimore R. Neonatal nosocomial infections. Semin Perinatol 1998;22:25–32.
[20] Harris J, Goldmann D. Infections acquired in the nursery: epidemiology and control. 2001.
[21] Stoll BJ, Gordon T, Korones SB, et al. Late-onset sepsis in very low birth weight neonates: a report from the National Institute of Child Health and Human Development Neonatal Research Network. J Pediatr 1996;129: 63–71.
[22] Chiesa C, Panero A, Osborn JF, et al. Diagnosis of neonatal sepsis: a clinical and laboratory challenge. Clin Chem 2004;50:279–87.
[23] Baker C. Group B streptococcal infections. Clin Perinatol 1997;24: 59–70.
[24] Grella M. C-reactive protein to determine the duration of antibiotic therapy in infants with suspected sepsis. Pediatrics 1997;100:900–1.
[25] Delanghe J, Speeckaert M. Translational research and biomarkers in neonatal sepsis. Clin Chim Acta 2015;451(Pt A):46–64.
[26] Hsu C, Cao H, Zhen H. Pathogenic bacteria distributions and drug resistance analysis in 96 cases of neonatal sepsis. BMC Pediatr 2017;17:44–9.
[27] Jean-Baptiste N, Benjamin D, Cohen-Wolkowiez M, et al. Coagulase-negative staphylococcal infections in the neonatal intensive care unit. Infect Control Hosp Epidemiol 2011;32:679–86.
[28] Shafer EM, Kumar P, Dutta S, et al. Blood culture confirmed bacterial sepsis in neonates in a North Indian tertiary care center: changes over the last decade. Jpn J Infect Dis 2009;62:46–50.
[29] Shane AL, Stoll BJ. Neonatal sepsis: progress towards improved diagnosis. J Pediatr 2014;68(Suppl 1):S24–32.
[30] Le Doare K, Heath PT. An overview of global GBS epidemiology. Vaccine 2013;31(Suppl 4):D7–12.
[31] Di Renzo GC, Melin P, Berardi A, et al. Intrapartum GBS screening and antibiotic prophylaxis: a European consensus conference. J Matern Fetal Neonatal Med 2015;28:766–82.
[32] Villanueva-Uy ME, Wongsmireel P, Sangtawesin V, et al. The burden of invasive neonatal group b streptococcal (gbs) disease in thailand and the philippines. Southeast Asian J Trop Med Public Health 2015;46: 728–37.
[33] Lan P, Li-qiang C, Da-li C, et al. Perinatal GBS colonization and trend of neonatal early-onset group B streptococcal disease (Chinese). Chin J Woman Child Health Res 2016;27:26–8.
[34] Yang M-J, Sun P-L, Wen K-C, et al. Prevalence of maternal group B streptococcus colonization and vertical transmission in low-risk women in a single institute. J Chin Med Assoc 2012;75:25–8.
[35] Choker A, Watser D, Eleaume H, et al. Correlation between biofilm formation and production of polysaccharide intercellular adhesin in clinical isolates of coagulase-negative staphylococci. Int J Med Microbiol 2006;296:381–8.
[36] Otto M. Molecular basis of Staphylococcus epidermidis infections. Semin Immunopathol 2012;34:201–14.
[37] Anday E, Talbot G. Coagulase-negative Staphylococcus bacteremia—a rising threat in the newborn infant. Ann Clin Lab Sci 1985;15: 246–51.
[38] Viswanathan R, Singh AK, Mukherjee S, et al. Aetiology and antimicrobial resistance of neonatal sepsis at a tertiary care centre in eastern India: a 3 year study, Indian J Pediatr 2011;78:409–12.
[39] Roy S, Viswanathan R, Singh A, et al. Gut colonization by multidrug-resistant and carbapenem-resistant Acinetobacter baumannii in neonates. Eur J Clin Microbiol Infect Dis 2010;29:1495–500.
[40] Al-Taiar A, Hammond MS, Cuing L, et al. Neonatal infections in China, Malaysia, Hong Kong and Thailand. Arch Dis Child Fetal Neonatal Ed 2013;98:F249–255.
[41] Russell A, Sharland M, Heath P. Improving antibiotic prescribing in neonatal units: time to act. Arch Dis Child Fetal Neonatal Ed 2012;97: F141–146.
[42] Muller-Pebody B, Johnson A, Heath P, et al. Empirical treatment of neonatal sepsis: are the current guidelines adequate? Arch Dis Child Fetal Neonatal Ed 2011;96:F4–8.
[44] Ullah O, Khan A, Ambreen A, et al. Antibiotic sensitivity pattern of bacterial isolates of neonatal septicemia in Peshawar, Pakistan. Arch Iran Med 2016;19:866–9.
[45] Pangercić A, Bukovski-Simonoski S, Barsić B. Lipopeptides and oxazolidinones–novel antibiotics in MRSA infection treatment. Lijec Vjesn 2010;132(Suppl 1):11–3.
[46] Gol C, Balmer P, Schwab F, et al. Different trends of MRSA and VRE in a German hospital, 1999–2005. Infection 2007;35:245–9.
[47] Astani A, Zimmermann S, Hassan E, et al. Antimicrobial activity of propolis special extract GH 2002 against multidrug-resistant clinical isolates. Pharmazie 2013;68:695–701.
[48] Viswanathan R, Singh AK, Basu S, et al. Multi-drug-resistant, non-fermenting, gram-negative bacilli in neonatal sepsis in Kolkata, India: a 4-year study. Paediatr Int Child Health 2014;34:56–9.
[49] Obiero CW, Seale AC, Berkley JA. Empiric treatment of neonatal sepsis in developing countries. Pediatr Infect Dis J 2015;34:659–61.
[50] Pammi M, Holland L, Butler G, et al. Candida parapsilosis is a significant neonatal pathogen: a systematic review and meta-analysis. Pediatr Infect Dis J 2013;32:e206–216.
[51] Clerihew L, McGuire W. Antifungal therapy for newborn infants with invasive fungal infection. Cochrane Database Syst Rev 2012;13:CD003953.