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

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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 ampicillin. 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 Staphycoccus aureus, NICU = neonatal intensive care unit, NS = neonatal sepsis, NT = not tested, PICC = peripherally inserted central catheter, PLT = platelet count, SMZ-TMP = Sulfamethoxazole-Trimethoprim, 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.[1] A consensus of the definition of neonatal sepsis has remained challenging.[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.[2,3] LONS, occurring after 72 hours from birth, is possibly due to bacteria transmitted from the hospital or the community during delivery.[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.[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.[6] China has

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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.[7]

The objective of this article was to investigate the bacterial pathogens, to analyze the associated risk factors, and to confer 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.[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). [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.[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 (BioMerieux La Balmes-les Grottes, France) by the Kirby Bauer disk diffusion method according to Clinical and Laboratory Standards Institution (CLSI) recommendations.[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 problems. 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, 3454 (13.14%) neonates were diagnosed with neonatal sepsis. Of 3454 neonates with clinical neonatal sepsis 976 (28%) and 2478 (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 abdominal distension (20.56%) occurred most commonly in LONS group. We analyzed the proportion of cases with abnormal hemogram in EONS and LONS groups respectively, and found that 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 n (%)	EONS n (%)	LONS n (%)	*P*
Gender				.81
Male	193 (56.60)	90 (55.90)	103 (57.22)	
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 (9.68)	**22 (13.70)**	11 (6.10)	.02*
Umbilical cord around neck	23 (6.45)	9 (5.60)	14 (7.80)	.42
1-min Apgar score	8.21 ± 2.06	7.83 ± 2.34	**8.80 ± 1.33**	.01*†
5-min Apgar score	8.85 ± 1.38	8.61 ± 1.50	**9.26 ± 1.04**	.03*†
Respiratory support	122 (35.78)	**98 (60.87)**	24 (13.33)	.00*
PICC/(UAC/UVC)	134 (39.30)	**100 (62.11)**	34 (18.89)	.00*
Duration of hospital stay (d)	20.50 (13.00, 50.25)	**42.5 (17.00,70.00)**	16.00 (11.00,27.00)	.00*
Antibiotic 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, PICC = peripherally inserted central catheter, UAC = umbilical artery catheter, UVC = umbilical venous catheter.

† Independent samples *t* test; No label: Mantel-Haenzel chi-square test or Fisher exact test.

* $P < .05$ means a significant difference between EONS and LONS groups.

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 n (%)	EONS (161) n (%)	LONS (180) n (%)	*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 (39.13)	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) $^{*}10^{9}$/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) * ng/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.

Table 3

The distribution of causative organisms in patients with early-onset and late-onset neonatal sepsis, 2013 to 2017.

Pathogenic microorganisms	Total n (%)	EONS (161) n (%)	LONS (180) n (%)
Gram-positive pathogens	215 (57.49)	87 (48.33)	**128 (65.98)**
CoNS	150 (69.77)	**63 (72.41)**	**87 (67.97)**
Staphylococcus haemolyticus	17 (11.33)	12 (19.05)	5 (5.75)
Staphylococcus epidermidis	78 (52.00)	28 (44.45)	**50 (57.47)**
Staphylococcus capitis	17 (11.33)	10 (15.87)	7 (8.05)
Staphylococcus hominis	20 (13.34)	6 (9.52)	14 (16.09)
Staphylococcus warneri	5 (3.33)	4 (6.35)	1 (1.15)
Other CoNS	13 (8.67)	3 (4.76)	10 (11.49)
Staphylococcus aureus	16 (7.44)	4 (4.60)	12 (9.38)
MSSA	10 (62.50)	0 (0)	**10 (83.33)**
MRSA	6 (37.50)	**4 (100.00)**	2 (16.67)
Enterococcus spp.	19 (8.84)	9 (10.35)	10 (7.81)
Enterococcus faecalis	9 (47.37)	5 (55.56)	4 (40.00)
Enterococcus faecium	10 (52.63)	4 (44.44)	6 (60.00)
Listeria spp.	1 (0.46)	1 (1.15)	0 (0)
Streptococcus spp.	29 (13.49)	10 (11.49)	19 (14.84)
Gram-negative pathogens	124 (33.15)	66 (36.67)	58 (29.90)
Klebsiella pneumoniae	31 (25.00)	14 (21.21)	17 (29.32)
ESBLs-	2 (6.45)	1 (7.14)	1 (5.88)
ESBLs+	29 (93.55)	13 (92.86)	16 (94.12)
Escherichia coli	33 (26.61)	7 (10.61)	**26 (44.83)**
ESBLs-	21 (63.64)	2 (28.57)	19 (73.08)
ESBLs+	12 (36.36)	5 (71.43)	7 (26.92)
Alcaligenes xylosoxidans	32 (25.81)	**27 (40.91)**	5 (8.62)
Serratia spp	2 (1.61)	2 (3.03)	0 (0)
Pseudomonas spp.	18 (14.52)	12 (18.18)	6 (10.35)
Acinetobacter spp.	1 (0.81)	0 (0)	1 (1.72)
Enterobacter spp.	5 (4.03)	2 (3.03)	3 (5.17)
Other gram-negative pathogens	2 (1.61)	2 (3.03)	0 (0)
Fungi	35 (9.36)	**27 (15.00)**	8 (4.12)
Candida guilliermondii	12 (34.29)	10 (37.04)	2 (25.00)
Candida pelliculosa	10 (28.57)	8 (29.63)	2 (25.00)
Other fungi	13 (37.14)	9 (33.33)	4 (50.00)

EONS = early-onset neonatal sepsis, ESBL = extended-spectrum beta-lactamase producer, LONS = late-onset neonatal sepsis, CoNS = coagulase-negative staphylococcus, MRSA = Methicillin-resistant Staphylococcus aureus, MSSA = Methicillin-sensitive Staphylococcus aureus.

group (40.91%). Fungal pathogens (3.5%) were relatively rare compared to bacteria, though they caused 27 (15%) of all EONS cases and 8 (4.12%) of all LONS cases. Among LONS group, *Staphylococcus epidermidis* followed by *E. coli* were the most common organisms, causing 50 (27.78%) and 26 (14.44%) of all LONS cases, respectively. Among EONS group, *S. epidermidis* followed by *A. xylosoxidans* were the most common organisms, causing 28 (17.39%) and 27 (16.77%) 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

Selected antimicrobial susceptibility patterns from the main gram-positive bacteria of septic neonates, 2013 to 2017.

Antibiotics	CoNS	*GBS*	*S. aureus*
Ampicillin/Sulbactam	31.76 (47/148)	NT	62.50 (10/16)
Benzylpencilline	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
Cefoxitin	28.79 (19/66)	NT	50.00 (2/4)
Ceftriaxone	NT	100.00 (19/19)	NT
Cefuroxime	30.87 (46/149)	100.00 (19/19)	62.50 (10/16)
Ciprofloxacin	51.11 (46/90)	NT	91.67 (11/12)
Clindamycin	54.24 (32/59)	30.00 (6/20)	50.00 (2/4)
Erythromycin	32.21 (48/149)	15.00 (3/20)	56.25 (9/16)
Furadantin	97.73 (43/44)	NT	100.00 (5/5)
Gentamicin	71.33 (107/150)	NT	93.75 (15/16)
Levofloxacin	56.38 (84/149)	90.00 (18/20)	93.75 (15/16)
Linezolid	99.29 (139/140)	100.00 (19/19)	100.00 (13/13)
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)
Fosfomycin	76.98 (97/126)	NT	84.62 (11/13)
Rifampin	86.00 (129/150)	NT	100.00 (16/16)
Teicoplanin	98.81 (83/84)	NT	100.00 (9/9)
Tigecycline	100.00 (109/109)	100.00 (5/5)	100.00 (15/15)
SMZ-TMP	57.72 (86/149)	NT	87.50 (14/16)
Vancomycin	98.65 (146/148)	100.00 (20/20)	100.00 (16/16)

CoNS = coagulase-negative staphylococcus, GBS = group B streptococcus, namely *S. agalactiae*, NT = not tested, SMZ-TMP = sulfamethoxazole-trimethoprim.

(Table 4). None of the 32 strains of *A. xylosoxidans* was resistant to tigecycline (100.00%), levofloxacin (100.00%), meropenem (100.00%), and cefoperazone/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.[5,11] 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.[4,12,13] 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 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/clavulanic acid	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/Sulbactam	75.00 (21/32)	36.36 (12/33)	8 (2/25)
Cefazolin	0.00 (0/32)	50.00 (16/32)	6.45 (2/31)
Cefepime	87.50 (28/32)	93.94 (31/33)	35.48 (11/31)
Cefmetazole	NT	100.00 (8/8)	25.00 (3/12)
Cefoperazone/sulbactam	100.00 (32/32)	96.97 (32/33)	22.58 (7/31)
Cefotaxime	NT	53.13 (17/32)	6.90 (2/29)
Cefotetan	3.13 (1/32)	100.00 (23/23)	69.23 (9/13)
Ceftazidime	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 (28/28)	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/tazobactam	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.

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.[14] Another 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.[15] 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.[16–18]

Early initiation of antimicrobial therapy is frequently delayed because the first clinical symptoms of sepsis are non-specific.[5] 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.[19] 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,[20–24] 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.[1] 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.[25] In our study, the proportion of abnormal neutrophil

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)
Voriconazole	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.[26] 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,[4,27] CoNS was the major Gram-positive pathogen for both EONS and LONS, which was consistent with previous studies in Asia and other developing countries.[19,28,29] 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[30–33] but very rare in China, which was consistent with other mainland China reports[34,35] 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.[36] CoNS infection is a major risk factor for premature infants.[19,27,28] 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.[37,38] 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, neonates 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 few recent studies reported *A. xylosoxidans* as one of the isolates.[29,39–41]*E. coli* (44.83%) followed by *Klebsiella spp.* (29.32%) was the most common Gram-negative bacteria in LONS. These findings were similar to other studies.[12]

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.[42] Currently, the recommended first-line therapy includes gentamicin+flucloxacillin and gentamicin+amoxicillin/penicillin.[43] 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 cartilage development, which made them an inappropriate choice for newborns.[44] 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.[45–47]*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[44]*Klebsiella spp.* and *E. coli* showed high sensitivity to meropenem, imipenem, and fosfomycin, and can be used as a first-line treatment.[12,48,49]

A recent study revealed that *Candida spp.* was responsible for 8 to 15% of hospital-acquired infections (HAIs).[50] 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.[30] The incidence rate of fungal infection in newborns was reported to be 10%,[51] 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 <1000 g.

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.

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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.

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