Newborns are prone to nosocomial infections because of their immature immune systems, and the risk increases with many factors such as preterm birth, low birth weight, vertical mother-to-infant transmission, perinatal infection, and the unreasonable use of antibiotics [1, 2]. In recent years, with the development of medical technology, the rescue success rate of premature infants and low-birth-weight infants has increased. As an important place for rescuing and treating newborns, the neonatal intensive care unit (NICU) has increased the risk of nosocomial infection outbreaks owing to the increase of invasive operations, the extension of hospital stay, and the application of broad-spectrum antibiotics. Studies have shown that the incidence of nosocomial infection in the NICU is 26.05%, and the location is mainly in the blood and lower respiratory tract [3]. Studies have also shown that neonatal sepsis is an important cause of death of children and newborns. Neonatal sepsis accounts for 7% of children's deaths and 16% of neonatal deaths [4].

It is therefore very important to identify the relevant pathogens and assess their drug resistance to reduce the rate of neonatal in-
fections. In this study, we statistically analyzed infection-related factors, bacterial distribution, and drug resistance in neonates treated at our hospital from January 2020 to June 2021. We compared the findings with the data for children and newborns from the national bacterial resistance surveillance report to provide a scientific basis for clinical formulation and evaluation of antimicrobial management policies.

Materials and Methods

Clinical samples
Clinical samples, including blood, sputum, cerebrospinal fluid, nasopharyngeal swab, and secretion samples, were taken from children admitted to the neonatology department of our hospital. For the analysis, the neonates were classified as having early-onset (within three days of birth) or late-onset (more than three days after birth) infection, being premature (born at < 37 gestational weeks) or term (born after 37 gestational weeks and < 42 gestational weeks), and having a low birth weight (weight < 2500 g) or normal birth weight (weight ≥ 2500 g).

Bacterial identification and drug resistance testing
Bacterial identification [5] and drug resistance testing were carried out using a French bioMérieux VITEK 2 Compact automatic bacterial culture identification instrument and its supporting drug susceptibility card. Antibacterial drug sensitivity was defined according to CLSI M100–2019.

National drug resistance surveillance data of children and newborn patients [6]
These data were obtained from "Research on Bacterial Resistance Surveillance in Children and Newborn Patients in China from 2014 to 2017" Chinese Medical Journal, Vol. 98, No. 40, October 30, 2018.

Statistical analysis
WHONETS.6 and SPSS22.0 were used to analyze the data. Data are expressed as percentages, and the chi-square test was used for between-group comparisons. When the frequency of a listed item was < 1, Fisher’s exact probability method was used to compare the data. Statistically significant differences were defined as P < 0.05.

Results

Bacterial survey
Patient characteristics
From January 2020 to June 2021, the Department of Neonatology at our hospital requested bacterial culture analysis of 4,572 samples from neonates. A total of 209 strains were isolated, for a positive culture rate of 4.57%. Among the 209 culture-positive patients, 14 died and 195 were discharged healthy. Of the 14 deaths, 8 were ultra-premature infants due to respiratory failure, and 6 were due to neonatal sepsis. In all, 119 were male (56.9%) and 90 were female (43.1%). The minimum age was 1 day, the maximum age was 3 months, and the average age was 19.3 days. There were 81 cases of vaginal delivery (38.8%) and 128 cases of cesarean section (61.2%); 29 cases (13.9%) had early onset of infection, and 180 cases (86.1%) had late onset of infection; 184 of the infants (88.0%) were born prematurely, and 25 (12.0%) were full-term infants. One hundred and seventy-seven infants (84.7%) had low birth weight, and 32 infants (15.3%) had normal birth weight (Table 1).

Specimen types
The 209 culture-positive cases were identified from 97 blood samples, 64 were from sputum samples, 15 were from cerebrospinal fluid samples, 25 were from nasopharyngeal swabs, and 8 were from secretions.

Bacterial types and distribution
Among the 209 bacterial isolates, 90 (43.1%) were gram-positive. The most commonly isolated bacteria were coagulase-negative Staphylococcus (42.2%), Staphylococcus aureus (32.1%), Enterococcus (13.3%), and Streptococcus agalactiae (4.5%). Data from the children and newborn groups of the national drug resistance surveillance report indicate [1] that the top gram-positive bacteria are mainly Staphylococcus aureus (35.6%), Streptococcus pneumoniae (27.4%), coagulase-negative Staphylococcus (21.1%), and Enterococcus faecium (4.4%) (Table 2).

Gram-negative bacteria were isolated from 119 cases (56.9%). Among them, the five most common isolates were Klebsiella pneumonia (56.3%), Acinetobacter baumannii (15.1%), Enterobacter aerogenes (7.6%), Enterobacter cloacae (5.9%), and Serratia marcescens (5.9%). Data from the National Drug Resistance Surveillance Children and Newborn Group report show that the top five gram-negative bacteria are Escherichia coli (26.8%), Klebsiella pneumoniae (16.8%), Haemophilus influenzae (15.5%), Pseudomonas aeruginosa (6.4%), and Acinetobacter baumannii (6.2%) (Table 3).

Distribution of pathogenic bacteria in children with early- or late-onset infection
In total, 27 pathogenic bacteria, seven gram-negative bacteria, and 20 gram-positive bacteria were detected in neonates with early onset of infection. Also, 182 pathogenic bacteria, 112 gram-negative bacteria, and 70 gram-positive bacteria were detected in the late-onset group. The pathogenic bacteria distribution between the two groups was not statistically significant, except for Klebsiella pneumoniae and Streptococcus agalactiae (P < 0.05).

Distribution of pathogenic bacteria in children with different gestational ages
In total, 184 strains of pathogenic bacteria, 108 gram-negative strains, and 76 gram-positive strains were detected in preterm infants, and 25 strains of pathogenic bacteria, 11 gram-negative strains, and 14 gram-positive strains, were detected in the term infant group. The pathogenic bacteria distribution between the two groups was not statistically significant, except for Streptococcus agalactiae (P < 0.05).
Distribution of pathogenic bacteria in children with different birth weights

In total, 172 pathogenic bacteria, 100 gram-negative strains and 72 gram-positive strains were detected in children with low birth weight; and 27 pathogenic bacteria, nine gram-negative bacteria and 18 gram-positive bacteria were detected in children with normal body weight. The pathogenic bacteria distribution between the two groups was not statistically significant, except for Streptococcus agalactiae (P < 0.05).

Comparison of EOS and LOS infections in preterm and term infants

We conducted a stratified analysis of EOS and LOS in preterm and term infants. We found that the number of LOS cases in preterm infants was much larger than that in other categories, with a P-value of <0.01, indicating a statistically significant difference. We found that premature infants require long-term parenteral nutrition and long, invasive procedures owing to immature organ development, poor skin and mucosal barrier function, and low humoral and cellular immunity, which are risk factors for LOS. Some studies have found that hospitalization stays ≥ 10 days is a risk factor for nosocomial infection [3], and the results of this study are consistent with it (Table 4).

Antimicrobial resistance of the main bacterial isolates

Staphylococcus aureus resistance

In this study, 35 strains of Staphylococcus aureus were isolated, of which 22 (62.9 %) were methicillin-resistant Staphylococcus aureus (MRSA). The 22 MRSA strains were sensitive to linezolid, vancomycin, rifampicin, levofloxacin, and gentamicin and were resistant to compound trimethoprim, clindamycin, and erythromycin (Table 5). The rate of methicillin resistance that we observed in our hospital was lower than that reported at the national level.

Streptococcus agalactiae resistance

Five Streptococcus agalactiae strains were isolated in this study. The strains were sensitive to most antibacterial drugs, with a resistance rate of 100 % to clindamycin and 60 % to tetracycline.

### Table 1

Basic characteristics of the 209 culture-positive neonates.

| Project                  | Classification      | Number of cases | Proportion % |
|--------------------------|---------------------|-----------------|--------------|
| Sex                      | Male                | 119             | 56.9         |
|                          | Female              | 90              | 43.0         |
| Delivery method          | Vaginal delivery    | 81              | 38.8         |
|                          | Cesarean section    | 128             | 61.2         |
| Onset time               | Early onset         | 29              | 13.9         |
|                          | Late onset          | 180             | 86.1         |
| Gestational age          | Premature baby      | 184             | 88.0         |
|                          | Full-term child     | 25              | 12.0         |
| Birth weight             | Low birth weight    | 177             | 84.7         |
|                          | Non-low birth weight| 32              | 15.3         |

### Table 2

Distribution of the four most common gram-positive bacterial isolates.

| Bacteria species            | Ratio (%) | Bacteria species            | Ratio (%) |
|-----------------------------|-----------|-----------------------------|-----------|
| 1 Coagulase-negative Staphylococcus | 42.2      | Staphylococcus aureus       | 35.6      |
| 2 Staphylococcus aureus     | 32.1      | Streptococcus pneumoniae    | 27.4      |
| 3 Enterococcus             | 13.3      | Coagulase-negative Staphylococcus | 21.1      |
| 4 Streptococcus agalactiae | 4.5       | Enterococcus faecium        | 4.4       |

### Table 3

Distribution of the five most common gram-negative bacterial isolates.

| Rank | Bacteria species | Ratio (%) | Bacteria species | Ratio (%) |
|------|------------------|-----------|------------------|-----------|
| 1    | Klebsiella pneumoniae | 56.3      | Escherichia coli | 26.8      |
| 2    | Acinetobacter baumannii | 15.1      | Klebsiella pneumoniae | 16.8      |
| 3    | Enterobacter aerogenes | 7.6       | Haemophilus influenzae | 15.5      |
| 4    | Enterobacter cloacae | 5.9       | Pseudomonas aeruginosa | 6.4       |
| 5    | Serratia marcescens | 5.9       | Acinetobacter baumannii | 6.2       |
Klebsiella pneumoniae resistance
In total, 67 Klebsiella pneumoniae strains were isolated in this study, and the drug sensitivity results (▶ Table 6) showed that the rates of sensitivity to amikacin, ertapenem, and imipenem were all 100%.

Acinetobacter baumannii resistance
Eighteen strains of Acinetobacter baumannii were isolated in this study. The drug sensitivity results were compared with national drug resistance monitoring data (▶ Table 7). One hundred percent of the bacterial isolates from our hospital were sensitive to tobramycin, imipenem, and gentamicin.

Discussion
Neonatal infections, especially the likes of neonatal sepsis, pneumonia, or meningitis, are major diseases that threaten newborn lives. Early diagnosis of these diseases is difficult. Bacterial culture is the “gold standard” for diagnosis. Strain identification and drug sensitivity tests provide a scientific basis for guiding rational drug use and controlling infection [7].

This study shows that, from January 2020 to June 2021, the rate of positive bacterial cultures in the samples taken by the neonatology department of our hospital was 4.35%, which is lower than that reported in the national drug resistance monitoring data. This is most likely because of regional differences. Most of the infections detected in newborns delivered at our hospital were acquired in the intrauterine environment; the second most common cause of nosocomial infections was prolonged hospitalization. In contrast, most of the children and neonates reported by the National Drug Resistance Surveillance Network exhibited community-acquired infections. Furthermore, gram-positive bacteria accounted for 43.1% and gram-negative bacteria for 56.9% of isolates in this study, virtually the same as the 45.5% gram-positive and 54.5% gram-negative rates reported by the national drug resistance monitoring network.
This study conducted a stratified analysis of EOS and LOS in preterm infants and term infants, finding that the number of LOS cases in preterm infants was much greater than the number of cases in other categories, with a P-value of <0.01, indicating a statistically significant difference. A possible explanation for this is that premature infants and low birth weight infants undergo more invasive procedures, such as intravenous catheterization and mechanical respiratory support during medical treatment, are treated for a longer time, and receive more antibiotics, increasing their risk of nosocomial infection. This is consistent with the fact that the rate of late-onset infection is significantly higher than that of early-onset infection in our study. According to expert consensus on the diagnosis and management of neonatal sepsis (version 2019), EOS patients were treated with a broad-spectrum combination of antibiotics before blood culture and other non-specific test results. Ampicillin (or penicillin) and third-generation cephalosporin were used as the first-line antimicrobial combination for gram-positive (G+) and gram-negative (G-) bacteria as early as possible. LOS patients were treated with phenazacillin and nafcillin for staphylococcus epidermidis or vancomycin instead of ampicillin combined with third-generation cephalosporin. The follow-up treatment plan should be adjusted according to the results of the drug susceptibility test, and in principle, priority is given to treatment with antibiotics alone rather than in combination. If the antibiotics selected are not empirically in the range of the drug susceptibility test and the clinical effect is good, they will continue to be used; otherwise, they will be changed to the sensitive antibiotics according to the drug susceptibility test results.

Our study shows that Streptococcus agalactiae (GBS) is one of the top four gram-positive bacteria isolated at our hospital. This is consistent with the fact that our hospital is a specialist hospital for obstetrics and gynecology, given that GBS normally resides in the vagina and intestines, and newborns can acquire the infection vertically from the mother. Early-onset GBS infections primarily cause pneumonia, meningitis, and sepsis [8, 9]. Studies [10, 11] have shown that GBS is highly sensitive to penicillin, ampicillin, cephalosporins, and vancomycin, and the drug sensitivity tests that we performed yielded similar results. Therefore, for children with early-onset GBS infection, penicillin is the first choice for treatment; for those with mild or severe allergies to penicillin, cefazolin or clindamycin can be used, respectively; and for those who are resistant to clindamycin, vancomycin should be used. Doctors perform skin sensitivity tests on patients before they are given penicillin drugs to prevent allergic reactions to penicillin. If the test is negative, penicillin is given to the patient; otherwise, it is prohibited for those who are positive. Of the 209 patients in this study, 20 did not undergo the penicillin skin sensitivity test, so the results are unknown. The skin test results of the remaining 189 patients were negative. Among the gram-negative bacteria isolated in this study, 18 strains were Acinetobacter baumannii. This is likely because of the frequent use of mechanical ventilation in neonatal intensive care, which increases the incidence of ventilator-associated pneumonia. Acinetobacter baumannii is the most common pathogen responsible for ventilator-associated pneumonia [12, 13].

Two of the top five gram-positive bacteria and gram-negative bacteria reported by the national drug resistance surveillance data, Streptococcus pneumoniae and Haemophilus influenzae, were not identified in this study. This may be related to differences in the study populations because these bacteria tend to circulate in the community and are not often the main cause of bacterial infection in neonatal patients.

In this study, 67 strains of Klebsiella pneumoniae were detected, of which 58 strains were extended-spectrum β-lactamase (ESBL) strains. Strains can acquire ESBL activity through bacterial plasmids, enabling them to hydrolyze broad-spectrum penicillins, cephalosporins, and monocyclic antibiotics (aztreonam), leading to increased drug resistance [14]. The drug sensitivity results showed a sensitivity rate of 100 % to all antimicrobial agents tested, except for amikacin, ertapenem, and imipenem, to which the isolates exhibited varying degrees of resistance.

In summary, newborns are susceptible to a wide range of bacterial infections and complex risk factors. There are differences in bacterial distribution and drug resistance in different regions and age groups. Therefore, understanding the distribution and drug resistance of pathogens in our hospital is greatly significant for guiding the rational selection of antibiotics in clinical practice and reducing neonatal mortality and nosocomial infections.

### Conflict of Interest

The authors declare that they have no conflict of interest.

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