

Original Article

Antibiotic Susceptibility Patterns in the NICU of Ghaem Hospital of Mashhad

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Background and Aims: Neonatal sepsis is considered a clinical syndrome characterized by signs and symptoms of infection associated with positive blood culture. The present study investigates the rate of sensitivity and resistance to antibiotics in neonates with definite sepsis.

Materials and Methods: This cross-sectional study was conducted on 268 neonates with definitive sepsis (positive blood culture with clinical signs of infection) hospitalized in the NICU of Ghaem Hospital of Mashhad, from 2008 to 2018. To investigate the antibiotic susceptibility pattern, identifying microorganism and antibiogram tests was performed according to the standard microbiological method. The data were collected through a questionnaire designed by the researchers. It included neonates' characteristics, types of microorganisms in neonatal unite, and sensitivity and resistance to neonatal sepsis's common microorganism.

Results: Based on the results, *Klebsiella* showed sensitivity to norfloxacin (100%), ciprofloxacin (100%), meropenem (100%), imipenem (94%), co-trimoxazole (73%), and vancomycin (67%). Similarly, *Enterobacter* showed 100% sensitivity to ciprofloxacin, meropenem, norfloxacin, and high sensitivity to imipenem (94%) and co-trimoxazole (83%). *Acinetobacter* turned out to be sensitive to co-trimoxazole and norfloxacin (both of them were 67%) and to amikacin in 33% of the cases. *E. coli* was sensitive to imipenem (83.33%), ciprofloxacin (80%), and ceftazidime (71.43%). Finally, *staphylococcus* coagulase negative was sensitive to piperacillin in 100%, vancomycin in 96.67%, and imipenem in 71.43% of the cases.

Conclusions: The findings of the present study suggest that high-sensitivity drugs for the treatment of definite neonatal sepsis are Meropenem(*Klebsiella* and *E. coli*), *Enterobacter* (Ampicilin), *Acinetobacter* (Imipenem) and *Staphylococcus* coagulase negative (vancomycin).

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Introduction

Neonatal sepsis is a life-threatening condition that may lead to mortality if not treated promptly and appropriately [1]. As a clinical syndrome, it is characterized by signs and symptoms of infection associated with positive blood culture [1, 2]. Septicemia is a global problem and, despite the presence of highly effective antibiotics and extensive health care, it is still one of the major causes of mortality and morbidity in infants, especially in countries with limited facilities [3, 4]. In a study, about 27% of neonatal mortality was due to infection [5]. Accordingly, on-time diagnosis and identification of pathogens are of high importance [6].

Although the clinical diagnosis of sepsis is difficult due to the non-specificity of the symptoms and signs of the disease [7], the combination of clinical signs and symptoms associated with positive blood culture is considered a golden standard for diagnosing neonatal sepsis [8]. Neonatal infections' signs and symptoms include fever, lethargy, restlessness, poor breastfeeding, respiratory distress, cyanosis, pallor, hypothermia, vomiting, tachycardia, and abdominal distention [9]. The cause of neonatal sepsis and its response to antibiotics can vary from time to time and place to place, affecting experimental treatments' effectiveness [10]. Selecting the proper antibiotic in suspected infections is often a severe challenge to neonatologists. To select an appropriate antibiotic, it is necessary to identify the bacterial colonized in the maternal genital tract

(in early sepsis), the neonatal intensive care unit (NICU)'s prevalent micro-organisms, and the personnel' hands. It is also essential to examine the sensitivity of these micro-organisms to antibiotics used in the NICU. Several studies have been carried out on the bacterial colonization in the maternal genital tract [11, 12] and neonatal wards in our country [13-15]. However, little information is available on the sensitivity and resistance of antibiotics neonatal sepsis. Because of the lack of proper and on-time treatment, neonatal sepsis is considered a life-threatening disease, and determining the antibiotic sensitivity pattern of the bacteria is essential for adequate treatment of neonatal sepsis [4]. It is better to elucidate the empirical antibiotic therapy in patients with sepsis. The present study aimed to investigate the antibiotic resistance and sensitivity to the common microorganism of definitive sepsis in Ghaem Hospital of Mashhad from 2008 to 2018.

Materials and Methods

In this cross-sectional study, out of 5426 neonates referred to the NICU of Ghaem Hospital from 2008 to 2018, 268 cases with definitive neonatal sepsis were selected. Patients with incomplete data were excluded from the study. The data collection tool was a researcher-made checklist of the types of microorganisms in the neonatal unite and sensitivity and resistance to common microorganisms in neonatal sepsis. In neonatal evaluation, age at onset of sepsis and gestational age were recorded. Neonates with

suspected sepsis were investigated for bacterial etiologic agents. Diagnosis of sepsis is defined based on positive blood culture plus clinical symptoms or signs. Blood samples were taken in admitted newborns with sterile conditions before the onset of the experimental treatment. Neonatal blood samples were aseptically collected by neonatal nursing before the antibiotic therapy and were sent to the Ghaem Hospital microbiology laboratory to identify isolates by Gram stains and culture growth. Positive blood cultures were sub-cultured onto blood, chocolate, and Mac Conkey agar plates and incubated at 37°C for 24 hours. This study's culture medium was BHI Broth M210-500G made by the Indian company of HiMedia. Quantitative cultures were not performed. Antimicrobial susceptibility testing was done by a Kirby-Bauer disc diffusion method following overnight incubation on Muller-Hinton agar plates. This study results from an approved Mashhad University of Medical Sciences project by the number of 960925, IR.MUMS.fm.REC.1396.587.

Statistical analysis

After data collection, the data were analyzed using SPSS version 21 and described using mean, standard deviation, and frequency.

Results

Out of 5436 infants' blood cultures, 268 were reported positive. Mean \pm SD of the age of onset of sepsis was 9.55 ± 8.94 days and of the gestational age was 31.33 ± 4.41 weeks. Fifty-eight cases were positive in the first blood

culture and 210 cases in the second. As illustrated in Figure 1, the types of microorganisms included 65 (24.1%) cases of *Klebsiella pneumonia*, 53 (19.6%) cases of *Staphylococcus epidermis*, 43 (16.3%) cases of *Enterobacter*, 27 (10%) cases of *E. coli*, 16 (5.9 %) cases of *Staphylococcus saprophyticus*, 14 (5.2%) cases of *Acinetobacter*, 11 (4.1%) cases of coagulase-negative staphylococci, 8 (3%) cases of *Staphylococcus aureus*, 6 (2.2%) cases of *Pseudomonas*, and 25 (9.6%) cases of other bacterial (*Enterococcus*, gram-negative bacilli, *Enterococcus faecalis*, *Streptococcus*, *Klebsiella rhinoscleromatis*, *Klebsiella oxytoca*, *Citrobacter* and alpha-hemolytic streptococcus). *Klebsiella* was found to be sensitive to norfloxacin, ciprofloxacin, and meropenem in 100% of cases. Also, the sensitivity of *Klebsiella* to imipenem was 93.94%, cotrimoxazole 72.73%, vancomycin 66.67%, ceftazidime 46.67%, cefoxitin 35.29%, amikacin 19.05%, cefixime 17%, and cefotaxime was 14.29%. *Klebsiella* was resistant to amoxicillin and gentamicin in 100% of cases (Fig. 2). *Enterobacter* was sensitive to cefoxitin, ampicillin, ciprofloxacin, meropenem, norfloxacin, and piperacillin in 100% of cases. The sensitivity of *Enterobacter* to imipenem, cotrimoxazole, ceftazidime, and amikacin was 94.12%, 83.33%, 35.29%, and 16.67% of the cases, respectively. *Enterobacter* was resistant to ceftizoxime 87.5%, cefotaxime 90%, gentamicin, ceftriaxone, cefalotin, cefixime, and ceftazolin in 100% of cases (Fig. 3).

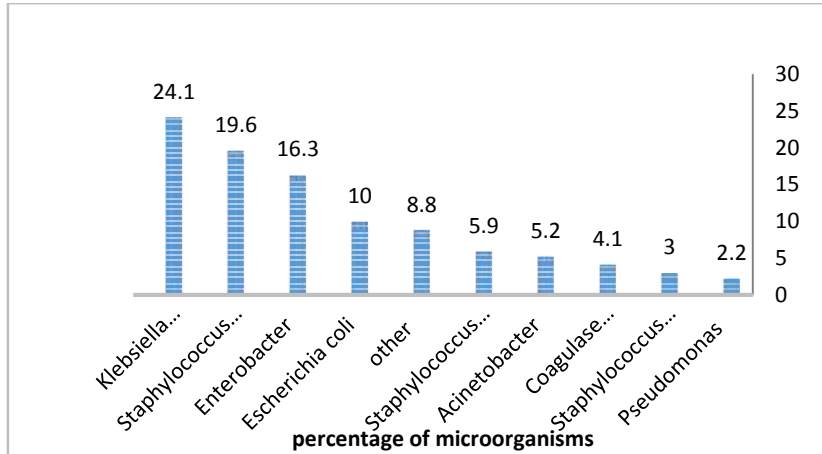


Fig. 1. Types of microorganisms at neonatal sepsis

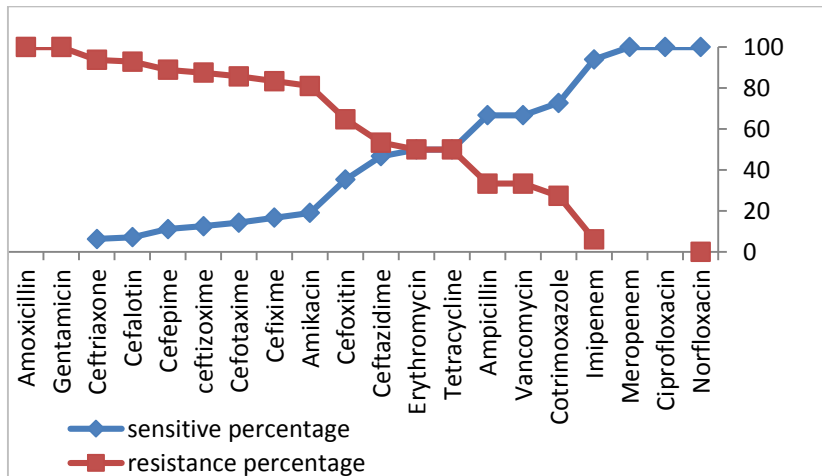


Fig. 2. Sensitivity and resistance of *Klebsiella* to antibiotics

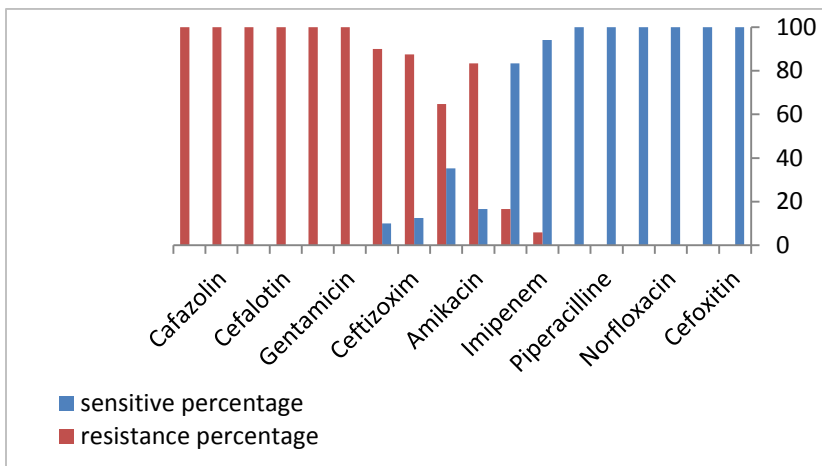


Fig. 3. Sensitivity and resistance of *Enterobacter* to antibiotics

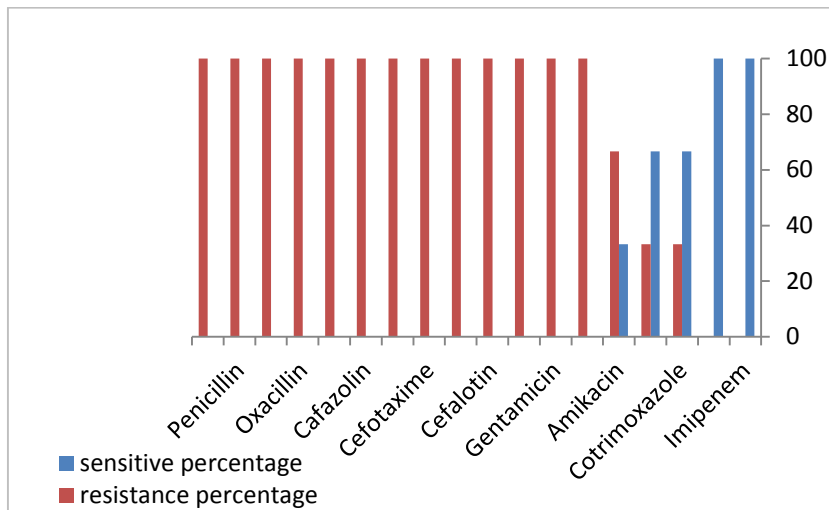


Fig. 4. Sensitivity and resistance of *Acinetobacter* to antibiotics

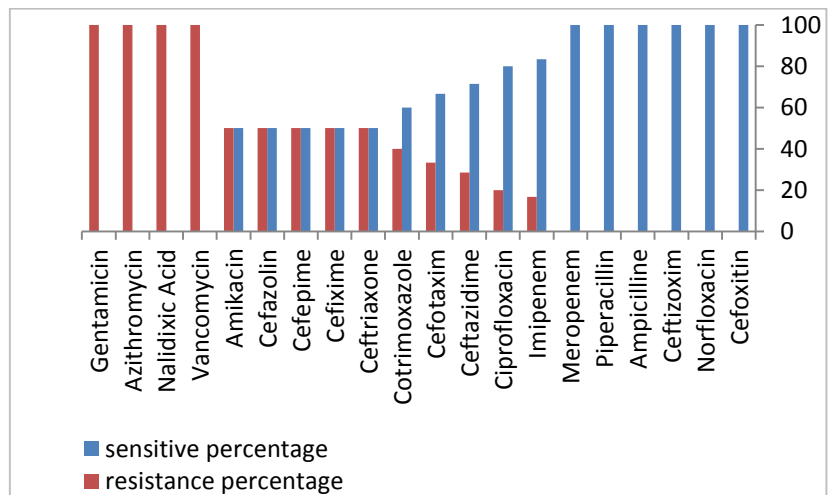


Fig. 5. Sensitivity and resistance of *E. coli* to antibiotics

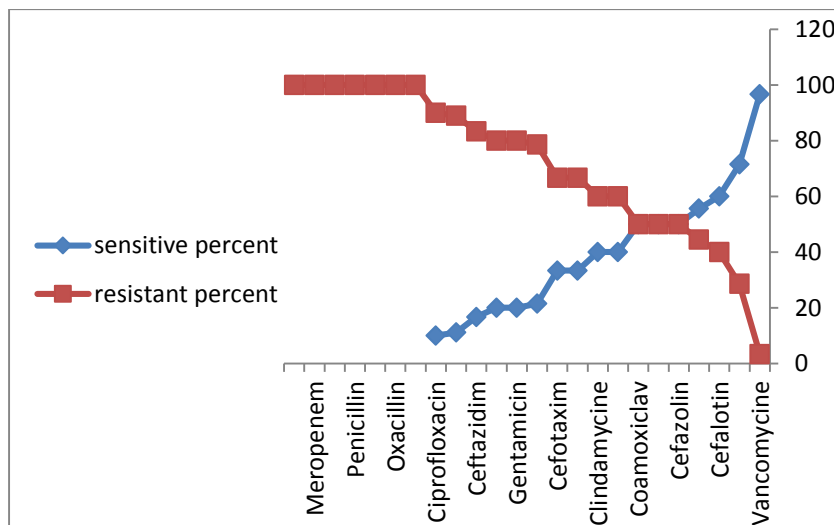


Fig. 6. Sensitivity and resistance to *Staphylococcus coagulase negative*

Acinetobacter was sensitive to imipenem in 100%, co-trimoxazole and norfloxacin 66.67%, and amikacin 33.33% of cases. In 100% of cases, *Acinetobacter* was resistant to cefixime, erythromycin, penicillin, gentamicin, ceftriaxone, amoxicillin, cefalotin, cefoxitin, cefotaxime, co-amoxiclav, cefazolin, ampicillin, oxacillin, and vancomycin (Fig. 4). *E. coli* was sensitive to meropenem, piperacillin, ampicillin, ceftizoxime, norfloxacin, cefoxitin 100%, imipenem 83.33%, ciprofloxacin 80%, cefotaxime 66.67%, co-trimoxazole 60%, ceftriaxone and cefixime in 50% of cases. Also, in 100% of cases, there was resistance to gentamicin, azithromycin, and vancomycin (Fig. 5).

Staphylococcus coagulase negative was sensitive to piperacillin in 100%, vancomycin 96.67%, and imipenem 71.43% of cases. There was resistance in 100% of cases to erythromycin, oxacillin, cefixime, penicillin, cefepime, meropenem, ampicillin, vancomycin, and azithromycin (Fig. 6).

Discussion

In the present study, the most common microorganisms in blood culture were found to be *Klebsiella pneumonia* (24.1%), *Staphylococcus epidermidis* (19.6%), *Enterobacter* (16.3%), *E. coli* (10%), and *Staphylococcus saprophyticus* (5.9%). The most common isolated bacteria in Prabhu et al. were *Staphylococcus aureus* (50.61%), *Staphylococcus* coagulase negative (12.3%), and *Klebsiella pneumonia* (12.3%) [3]. Sharif et al. (2000) reported that *Klebsiella* 35 (37.6%), coagulase-positive *staphylococci* 21 (22.5%),

coagulase-negative *staphylococci* 14 (15.05%), *E. coli* 14 (15.05%), *pseudomonas* 4 (4.3%), *enterobacter* 4 (4.3%), and *Serratia* 1 (1.07%) as the most commonly grown organisms [16]. In Ansari et al.'s study, the most common bacterial agents of sepsis were *Staphylococcus* coagulase negative and *Staphylococcus aureus* [17]. Muley et al. found *Klebsiella pneumonia* and *Staphylococcus aureus* to be the most common neonatal sepsis pathogens [18]. Mythri et al. (2016) studied the most common neonatal sepsis pathogens, including *Klebsiella*, *Staphylococcus* coagulase negative, *Staphylococcus aureus*, and gram-negative bacilli [2]. According to Pooja et al. (2015), the most common organisms of neonatal sepsis included *Klebsiella* (15.5%), *Staphylococcus aureus* (14.5%), *Enterobacter* (10.5%), and *Acinetobacter* (10.5%) [19]. In another study, the most common pathogens isolated from the patients of neonatal sepsis were *Klebsiella pneumoniae* (42%), followed by *Staphylococcus aureus* (17%), coagulase-negative *Staphylococcus* (14%), and *E. coli* (7%) [20]. The high contagion of the negative gram microbes in our department could result from the crowded wards, lack of enough space between the beds, inadequacy between the number of nurses and patients, and no hand washing by the personels. Neonatal wards microorganism showed significant sensitivity to vancomycin (97%), acceptable sensitivity to imipenem (72%), and relative sensitivity (about 50%) to co-trimoxazole, norfloxacin, cefalotin, and cefazolin. About two-thirds of cases were resistant to ampicillin, clindamycin, cefotaxime, and cefoxitin. Besides, about four-fifths of our

neonatal ward microorganisms were resistant to gentamicin, ceftizoxime, and ceftazidime. All microorganisms in our NICU were resistant to erythromycin, oxacillin, cefixime, and penicillin. In Shrestha et al. (2013), all microorganisms in the NICU, except the *Acinetobacter*, were sensitive to first-line antibiotics such as amikacin, gentamicin, cefotaxime, and ampicillin [21]. Ampicillin combined with gentamicin is the drug of choice for empirically treating neonatal early-onset sepsis [22]. The high resistance of microorganisms in our NICU to the first antibiotics such as ampicillin and gentamicin is a serious problem due to these agents' unusual use. It seems that the initiation of these antibiotics will not be effective in treating infants with high risk of infection, and a revision of these two drugs is necessary. The worrying result was the inappropriate sensitivity of microorganisms to cephalosporins, which tend to be the second antibiotic choice, in our NICU. In the present study, *Klebsiella* was very sensitive (> 94%) to meropenem and imipenem, and cotrimoxazole and vancomycin in two-thirds of the cases. The sensitivity rate of *Klebsiella* to ceftazidime was 47%, to ceftazidime 35%, to amikacin 19%, to cefixime 17%, and cefotaxime 14%. *Klebsiella* was resistant to amoxicillin and gentamicin in 100% of cases. A comparison of the findings with those of a prior study conducted ten years ago in the same center reveals an increase in the resistance of *Klebsiella* to cefotaxime from 54% to 86%, to amikacin from 18% to 81%, and gentamicin from 63% to 100%. This increasing resistance to our common antibiotics has developed at an alarming rate. In

their study, Sharif et al. reported that *Klebsiella*'s resistance to ampicillin was 15.5%, cloxacillin 23.8%, gentamicin 48.4%, and amikacin 3% [16]. In Khan et al. study, *Klebsiella* pneumonia showed the most sensitivity to amikacin (88.46%), meropenem (80.77%), ampicillin (76.92%), and ceftazidime (61.54%) [23]. The percentage of high resistance of *Klebsiella* to cephalosporins and aminoglycosides in this study was in contrast to previous studies, suggesting that these two large groups of antibiotics also do not affect the most common infectious microorganism in our wards and should be revised for their usual use.

Enterobacter was quite sensitive to ceftazidime, ampicillin, ciprofloxacin, meropenem, norfloxacin, piperacillin, and resistant gentamicin, ceftriaxone, cefalotin, cefixime, and cefazolin. In another study, gram-negative bacteria of Enterobacteriaceae were resistant to penicillins and cephalosporins with a broad spectrum. Therefore, using these antibiotics will not be effective alone [2].

Acinetobacter in two-thirds of cases was sensitive to co-trimoxazole and norfloxacin, and in one-third of the subjects showed sensitivity to amikacin. *Acinetobacter* was utterly resistant to cefixime, erythromycin, penicillin, gentamicin, ceftriaxone, and amoxicillin in our wards cephalothin, ceftazidime, cefotaxime, co-amoxiclav, cefazolin, oxacillin, and vancomycin. In Shrestha et al.'s (2013) study, *Acinetobacter* was sensitive to co-trimoxazole, azithromycin, cefotaxime, and ceftazidime [21].

E. coli was sensitive to imipenem and ciprofloxacin in four-fifth of cases, ceftazidime,

cefotaxime, and co-trimoxazole two-thirds of cases, and it showed resistance to tetracycline, gentamicin, azithromycin, and vancomycin. In Parajuli et al.'s study, all negative gram cocci were sensitive to amikacin [24]. *Staphylococcus coagulase negative* was completely sensitive to piperacillin and showed high sensitivity to vancomycin. In two-thirds of cases, sensitivity was observed to imipenem. In two-fifths of the cases, it was sensitive to amikacin and clindamycin, and in one-third of cases to ampicillin and cefotaxime. It showed a relative sensitivity to cephazolin, norfloxacin, and co-amoxiclav. There was high resistance to gentamicin, ceftizoxime, ceftazidime, ceftriaxone, and ciprofloxacin, and it was quite resistant to erythromycin, oxacillin, cefixime, penicillin, cefepime, meropenem, ampicillin, vancomycin, and azithromycin. *Staphylococcus coagulase-negative* is the main pathogen in late neonatal sepsis [25]. In the study of Zubair et al., *Staphylococcus coagulase negative* showed the highest sensitivity to vancomycin (97.7%), and amikacin (85.8%), and lower to co-amoxiclav (68.2%), ciprofloxacin (57.7%), ampicillin (44.6%), ceftriaxone (41.2%), amoxicillin (33%), oxacillin (24.2%), and penicillin (16%) [26]. In another study, the sensitivity of *Staphylococcus coagulase negative* was to amikacin (34%), penicillin (47%), and ceftriaxone (66%) [27].

To conclude, neonatal septic pathogens vary over time and even place [17]. Antibiotic

resistance is a global problem. The antibiogram pattern varies from country to country depending on the epidemiology of neonatal sepsis [28]. The difference in antibiotic use patterns in different hospitals is the main cause of various antibiotic sensitivities reported by different researchers [26].

Conclusion

In this study, the most common microorganisms of neonatal sepsis were *Klebsiella pneumonia*, *Staphylococcus epidermis*, *Enterobacter*, and *E. coli* *Staphylococcus saprophyticus*. The high resistance of microorganisms in our NICU to the first-line antibiotics such as ampicillin and gentamicin is a serious problem. These antibiotics do not seem to be effective in treating infants with a high risk of infection. The second challenging result is the inappropriate sensitivity of microorganisms to the third generation of cephalosporins, as the common second antibiotic choice, in our NICU. Our findings suggest that the high-sensitivity drugs for treating definite neonatal sepsis due to *Klebsiella* and *E. coli* are meropenem, acinetobacter imipenem, and *Staphylococcus coagulase negative* vancomycin.

Conflict of Interest

There is no conflict of interest to declare.

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