Risk Factors of Nosocomial Infection with Extended-Spectrum Beta-Lactamase-Producing Bacteria in a Neonatal Intensive Care Unit in China

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Abstract

Background: To study risk factors of neonatal nosocomial infection caused by extended-spectrum beta-lactamase (ESBL)-producing bacteria in a neonatal intensive care unit (NICU).

Patients and Methods: A retrospective cohort study was conducted in a university hospital NICU in south China. Medical records of neonatal nosocomial infection caused by *Escherichia coli* or *Klebsiella pneumoniae* were reviewed. Twenty-two neonates infected with ESBL-producing bacteria (case patients) were compared with 17 patients infected with non-ESBL producing strains (controls). Univariable and multivariable logistic regression were performed to analyze risk factors for infection with ESBL-producing strains. The spectrum of antimicrobial resistance of ESBL-positive *E. coli* or *K. pneumoniae* was also examined.

Results: Both univariable and multivariable logistic regression analysis revealed that preterm low birth weight, prolonged mechanical ventilation (≥ 7 days) and prior use of third-generation cephalosporins were risks factors for ESBL-producing *E. coli* or *K. pneumoniae* infection ($p < 0.05$), with an odd ratio of 6.43 (95% CI: 1.51–27.44; $p = 0.017$), 7.50 (95% CI: 1.38–40.88; $p = 0.017$) and 9.00 (95% CI: 1.65–49.14; $p = 0.008$) respectively. However, the length of hospital stay before isolation of pathogens, endotracheal intubation, presence of a central venous catheter, days on third-generation cephalosporins and prior use of beta-lactamase inhibitors were not statistically significant ($p > 0.05$). Resistance of ESBL-positive strains to piperacillin, tobramycin, aztreonam and cephalosporins was significantly higher than that of ESBL-negative ones ($p < 0.05$). ESBL-producing strains appeared susceptible to carbapenem, fluoroquinolones, and beta-lactamase inhibitor combination piperacillin-tazobactam.

Conclusions: Preterm low birth weight, prolonged mechanical ventilation and prior use of third-generation cephalosporins are risks factors for nosocomial infection with ESBL-producing bacteria in NICU.

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Introduction

Extended-spectrum beta-lactamase (ESBL)-producing bacteria were first isolated in Germany in 1983 [1]. Marked increase in the incidence of infections due to ESBL-producing organisms in recent years is of great concern [2–4]. ESBLs are beta-lactamases that hydrolyze extended spectrum cephalosporins with an oxyimino side chain and are most often associated with *Klebsiella pneumoniae*, but it is a plasmid-mediated trait that can transfer resistance to other Gram-negative bacilli, such as *Escherichia coli* and other enteric bacilli [5].

Because ESBL-producing organisms are frequently resistant to multiple antimicrobial agents, therapeutic options for these infections are limited and risk of treatment failure in patients infected with such strains is increasing. Extensive use of third generation cephalosporins is reported to be associated with increased prevalence of infection caused by ESBL-producing bacteria [6, 7]. Emergence of multi-drug resistant strains is an alarming problem in developing countries where the use of antibiotics is not strictly controlled. In China, antibiotics could be easily purchased over the counter without prescription until a few years ago. Furthermore, empirical use of third generation cephalosporins for infections is a very common practice in intensive care units, thus posing significant selective pressure on the resistant strains.

It is important to search for ways to minimize or eliminating risk factors that could lead to the emergence of multi-drug resistance of bacteria. Outbreaks caused by ESBL-producing organisms are well described in adults [8–11], but limited clinical data exist for neonates in neonatal intensive care units (NICU) particularly with

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respect to risk factors for infection. We therefore performed a retrospective study of nosocomial infection caused by ESBL-producing bacteria in NICU infants in a large university teaching hospital. The aim of the current study was to identify risk factors associated with ESBL-producing bacteria infection in NICU in an area with extensive empirical use of antibiotics.

Patients and Methods

Patients

The investigation was conducted at the NICU of the First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China, a 40-bed unit with an average annual admittance of – 800 high-risk neonates.

A retrospective review was performed on the records of clinical microbiology laboratory from January 2000 to December 2002 to identify neonatal nosocomial infection caused by *E. coli* or *K. pneumoniae* in NICU.

Clinical Data

We reviewed the medical records of neonatal nosocomial infection caused by *E. coli* or *K. pneumoniae*. The data collected included postnatal age, sex, gestational age, birth weight, APGAR scores, underlying disease, primary site of infection, duration of hospital stay before onset of infection, antimicrobial regimen, any antimicrobial therapy in the 2 weeks prior to onset of infection, and microorganisms identified in bacteriologic specimens and date of isolation. In addition, the presence of a central venous catheter or mechanical ventilation was assessed.

Definitions

Nosocomial bloodstream infection as well as other nosocomial infections, was defined according to the Centers for Disease Control and Prevention/National Nosocomial Infection Surveillance (NNIS) definitions for infants $\leq$ 12 months [12]. Nosocomial infection was defined as infection that occurred > 48 h after admission to NICU. Severity of illness was measured by the score for neonatal acute physiology [13].

Cases and Controls

Each patient was included as a case patient only once. If ESBL-producing *E. coli* or *K. pneumoniae* was isolated on multiple occasions, only the first episode of infection was reviewed. Potential controls were identified among hospitalized neonatal unit patients who were infected with non-ESBL-producing *E. coli* or *K. pneumoniae* during the same period. Designation as a case patient or a control patient was based solely on whether the infecting organism was identified to demonstrate ESBL resistance.

Potential risk factors for ESBL-producing *E. coli* or *K. pneumoniae* infection such as preterm low birth weight, prior administration of third-generation cephalosporins, length of exposure to third generation cephalosporins and β-lactams inhibitors, endotracheal tubes, central venous catheter, mechanical ventilation, number of hospital days prior to infection, and severity of illness were investigated.

Antimicrobial Therapy and Outcome of Patients

To evaluate the effect of ESBL-producing *E. coli* or *K. pneumoniae* infection on clinical outcome, initial response to treatment and 30-day mortality rate were assessed. The initial response to treatment was evaluated at 72 h after starting antimicrobial treatment and was defined according to the following criteria: (1) complete response – resolution of fever, leukocytosis and all signs of infection; (2) partial response – improvement but not complete resolution of the above parameters; (3) treatment failure – no improvement or even deterioration in any of these clinical parameters or death [14, 15]. The 30-day mortality rate was calculated as total number of deaths/total number of cases.

Empirical antimicrobial therapy was defined as the initial therapy before the results of culture were available. Appropriate antimicrobial therapy was defined if the infecting organism was subsequently found to be susceptible *in vitro* to the antibiotic administered and the dosage and route of administration were in conformity with current medical standards.

Mortality directly attributable to infection was defined as death in the setting of clinical evidence of active infection and a positive culture result.

Microbiological Methods

Isolates were sent for bacterial culture when infections were highly suspected. Clinical isolates from tracheal aspirates from endotracheal tubes, nasopharyngeal aspirates, blood, urine, and surgical wound were collected and cultured in the clinical microbiology laboratory in our hospital. Species identification was carried out with VITEK-GNI cards by standard methods [16]. Susceptibilities to all antimicrobial agents were determined by the disk diffusion method, employing the criteria of the National Committee for Clinical Laboratory Standards (NCCLS) [17]. Antimicrobial susceptibility test discs were supplied by OXOID Limited (Basingstoke, Hampshire, England). Antibiotics included in the susceptibility test were cefotaxime, ceftriaxone, ceftazidime, aztreonam, cefoxitin, amoxicillin-clavulanic acid, ciprofloxacin, norfloxacin, amikacin, gentamycin, tobramycin, ticarcillin-clavulanic acid, piperacillin, piperacillin–tazobactam, cefuroxime, cefoperazone, cefoperazone–sulbactam, imipenem, and meropenem. MICs were determined by the broth microdilution method, as described by NCCLS [18]. ESBL production was screened and determined by the disk diffusion method according to NCCLS performance standards. In brief, we determined the diameter of the inhibition zones on cefotaxime and ceftazidime disks (30 μg each), alone and in combination with clavulanic acid (10 μg). An increase of $\geq$ 5 mm in zone diameter when either of the antimicrobial agents was combined with clavulanic acid was confirmed as ESBL-positive (ESBL-P). Two control organisms, *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603, were inoculated in each set of tests for quality control.

Statistical Analysis

Statistical analysis was performed using SPSS11.0 statistical software. A p-value of less than 0.05 was considered statistically significant. Student t-test was used to compare continuous variables, and the χ^2 or Fisher exact test was used to compare categorical variables. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. In identifying the risk factors associated with development of ESBL-producing bacteria infection, a backward stepwise logistic regression analysis was used to control for the effects of confounding variables. Variables with a p-value of < 0.05 in the univariable analysis were candidates for multivariable analysis.

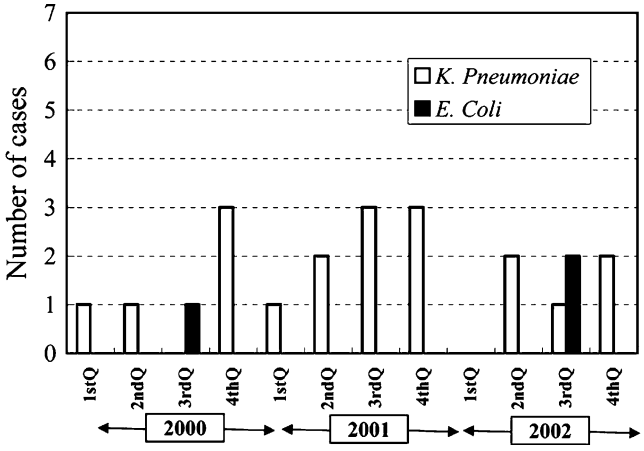

Figure 1. Neonates infected with ESBL-producing *K. pneumoniae* and *E. coli* in Neonatal Intensive Care Unit, First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China from January 2000 to December, 2002. 1stQ: First Quarter of the year; 2ndQ: second quarter; 3rdQ: third quarter; 4thQ: fourth quarter.

Results

Prevalence of Infection caused by ESBL-Producing Bacteria

Among 2,358 neonates admitted to the NICU during the study period, 19 (0.81%) were infected by ESBL-producing *K. pneumoniae* and 3 (0.13%) by ESBL-producing *E. coli.* Twelve (0.51%) were infected by non-ESBL-producing *K. pneumoniae* and 5 (0.21%) by non-ESBL-producing *E. coli*. All of them met criteria for infection [12]. ESBL-producing strains accounted for 56.4% of infections caused by *E. coli* or *K. pneumoniae.* The distribution of occurrence of infection caused by ESBL-producing *E. coli* or *K. pneumoniae* over time was shown in figure 1. There was no significant outbreak of infection caused by ESBL-producing bacteria.

Of the 22 patients with ESBL-producing bacteria infection, 19 (86.4%) had infection due to *K. pneumoniae* and 3 (13.6%) had infection due to *E. coli*. The sites of infection were as follows: respiratory in 12 patients (54.5%); urinary in 3 (13.6%); blood in 4 (18.2%); and surgical wound or other sites, in 3 (13.6%).

Risk Factors for ESBL-Producing *E. coli* or *K. pneumoniae* Infection

The general characteristics of case and control groups are shown in table 1. Case patients had significant lower birth weight and gestational age than the controls. In addition, a greater proportion of patients with ESBL-producing bacteria infection were preterm low birth weight infants.

Univariable analysis showed preterm low birth weight, prolonged mechanical ventilation (≥ 7 days) and prior use of third-generation cephalosporins were risk factors for ESBL-producing *E. coli* or *K. pneumoniae* infection (Table 2). Other variables including length of hospital stay before isolation of pathogens, endotracheal intubation, presence of a central venous catheter, days on third-generation cephalosporins and prior use of beta-lactamase inhibitors were not statistically significant ($p > 0.05$).

Using multivariable analysis with logistic regression, premature low birth weight, prolonged mechanical ventilation (≥ 7 days) and prior use of third-generation cephalosporins remained risk factors for ESBL-producing *E. coli* or *K. pneumoniae* infection (Table 3).

Antimicrobial Resistance of ESBL-Positive *E. coli* or *K. pneumoniae*

As shown in table 4, the resistance rates of ESBL-positive strains to piperacillin, tobramycin, aztreonam, and cephalosporins were significantly higher than that of ESBL-negative strains ($p < 0.05$). ESBL-positive strains appeared susceptible to carbapenem (imipenem, meropenem), fluoroquinolones (ciprofloxacin, norfloxacin), and beta-lactamase inhibitor combination piperacillin-tazobactam.

Clinical Conditions and Outcomes

The underlying conditions of 22 patients with ESBL-producing organisms infection were as follows: neonatal respiratory distress syndrome, 16 patients (72.7%); perinatal asphyxia, 2 (9.1%); meconium aspiration syndrome, 2 (9.1%); hemolytic disease of the newborn, 1 (4.5%); congenital anomalies of urinary tract, 1 (4.5%).

The underlying conditions of 17 control patients with non-ESBL-producing organism infection were as follows: neonatal respiratory distress syndrome, 7 patients (41.2%); perinatal asphyxia, 3 (17.6%); high risk neonates, 4 (23.5%); congenital heart disease, 1 (5.9%); neonatal hypoglycemia, 1 (5.9%); postsurgery of cleft lip and palate, 1 (5.9%).

Severity of illness was calculated by the score for neonatal acute physiology at admission. When the severity of illness was compared between the case and control groups, the difference was not statistically significant (Table 1).

Antibiotic treatment failed in 2 patients (9.1%), and these two patients died. The mortality was directly attributable to ESBL-producing *K. pneumoniae*. They received amoxicillin-clavulanic acid as empirical antimicrobial therapy when nosocomial infection occurred. In comparison, 20 (90.9%) of 22 case patients who survived received appropriate antimicrobial therapy with imipenem within 72 h of the time that the specimen was sent for culture. These 20 case patients had complete clinical response to therapy. The 30-day mortality rate of case group was 9.1%. Of the case survivors, 15% (3/20) had chronic lung diseases, 5% (1/20) had intraventricular hemorrhage grade III, 10% (2/20) had retinopathy of prematurity of stage III.

All 17 control patients had complete clinical response to antimicrobial therapy. The antibiotics used for

Table 1
General characteristics of neonatal patients in NICU.

Characteristics	Case patients (n = 22)	Control patients (n = 17)	*p*-value
Sex (male/female)	14/8	10/7	0.094
Birth weight (g)	2,055 ± 579	2,715 ± 1,086	0.019
Number (%) of low birth weight	18 (81.8%)	7 (41.1%)	0.017
Gestational age (week)	32.9 ± 3.1	36.1 ± 3.9	0.007
APGAR score (5 min)	7.8 ± 1.7	7.9 ± 1.5	0.816
Score for neonatal acute physiology at admission	6.3 ± 2.4	6.1 ± 3.2	0.809
Number (%) treated with antibiotics	21 (95)	16 (94)	1.00

Table 2
Potential risk factors for ESBL-producing *E. coli* or *K. pneumoniae* infection in NICU: results of univariable analysis.

Risk factors	Case patients (n = 22)	Control patients (n = 17)	OR (95% CI)	*p*-value
Preterm LBW	18	7	6.43 (1.51–27.44)	0.017
LOS before isolation of pathogens (days)	10.4 ± 6.5	8.1 ± 4.9	–	0.225
Endotracheal tube	16	7	3.81 (0.991–14.65)	0.059
Central venous catheter	8	3	2.67 (0.58–12.19)	0.288
Prolonged mechanical ventilation (≥ 7 days)	11	2	7.50 (1.38–40.88)	0.017
Prior use of 3GC	12	2	9.00 (1.65–49.14)	0.008
Days on 3GC (days)	8.3 ± 2.9	6.0 ± 1.4	–	0.190
Prior use of beta-lactamase inhibitors	8	9	0.508 (0.14–1.84)	0.345

LBW: low birth weight; LOS: Length of hospital stay; 3GC: third generation cephalosporins

Table 3
Results of multivariable analysis with logistic regression in a case-control study of risk factors for ESBL-producing *E. coli* or *K. pneumoniae* infection in NICU.

Variables	Coefficient	OR (95% CI)	*p*-value
Premature LBW	3.23	25.4 (1.8–348.8)	0.016
Prolonged mechanical ventilation (≥ 7 days)	3.15	23.5 (1.4–398.8)	0.029
Prior use of 3GC	2.55	12.8 (1.1–143.8)	0.039

LBW: low birth weight; 3GC: third generation cephalosporins

nosocomial infection were as follows: imipenem, 41.2% (7/17), cefoperazone-sulbactam, 35.3% (6/17), and amoxicillin-clavulanic acid, 23.5% (4/17). All control patients survived. Of all surviving control patients, 5.9% (1/17) had chronic lung diseases. No infants had severe intraventricular hemorrhage or retinopathy of prematurity.

Discussion

Nosocomial infection caused by ESBL-producing organisms is a growing concern in NICU, which is responsible for significant morbidity and mortality among high risk neonates. Because of the potential importance of ESBL-producing organisms in causing nosocomial outbreak of infections resistant to multiple antibiotics, identifying the risk factors for acquisition of ESBL-producing strains is critical. This study showed that preterm low birth weight, prolonged mechanical ventilation and prior use of third-generation cephalosporins are risk factors for ESBL-producing organism infection in NICU patients. This is in consistence with previous report that the risk of infection with ESBL-producing organism infection was associated with low birth weight, prolonged length of stay and empiric antibiotic treatment [19]. However, *Singh* et al. [20] reported that only very low birth weight and prolonged exposure to antimicrobial agents were independent risk factors associated with antimicrobial-nonsusceptible enterobacteriaceae infection. Our results identified prolonged mechanical ventilation as an additional risk factor. Moreover, we emphasized the importance of exposure to third-generation cephalosporins in the acquisition of ESBL-producing organisms.

In this survey, the main focus of ESBL-producing strains infection was pneumonia, and case patients had higher proportions of respiratory distress syndrome than did control patients (72.7% vs 41.2%). The majority of infants with respiratory distress syndrome received endotracheal intubation and mechanical ventilation soon after birth. One of the remarkable findings from this study was that prolonged mechanical ventilation was associated with ESBL-producing strains infection, whereas endotracheal intubation itself was not a statistically significant risk factor for infections with ESBL-producing strains. Therefore, interventions aimed at reducing the duration of mechanical ventilation can help to prevent infection

Table 4
Antimicrobial resistance of *E. coli* or *K. pneumoniae* isolated from a NICU (%).

Antibiotics	ESBL-positive strains	ESBL-negative strains	*p*
Amoxycillin/clavulanic acid	42.9	35.7	0.657
Ticarcillin/clavulanic acid	46.4	29.4	0.259
Piperacillin	96.4	68.7	0.010
Piperacillin-tazobactam	10.7	0	0.220
Gentamycin	35.3	41.1	0.714
Amikacin	60.7	43.7	0.277
Tobramycin	92.9	63.6	0.023
Cefuroxime	100.0	62.5	0.001
Cefoperazone	92.9	37.5	0.0003
Cefoperazone-sulbactam	27.8	0	0.119
Ceftriaxone	96.4	31.2	0.0001
Cefotaxime	89.3	35.2	0.0005
Ceftazidime	78.6	31.3	0.002
Aztreonam	89.3	31.3	0.0001
Imipenem	3.6	0	0.431
Meropenem	3.7	0	0.558
Ciprofloxacin	0	5.8	0.194
Norfloxacin	3.6	7.1	0.608

caused by ESBL-producing organisms in NICU patients. Moreover, mouth care, respiratory care, and enteral feeding of patients who receive prolonged mechanical ventilation are more likely to increase the risk of cross-infection by contact transfer. This pointed out the importance of hand transmission of ESBL-producing strains and the need of hand washing in NICU [21–23].

Third-generation cephalosporin is one of the most commonly used classes of antibiotics for hospitalized patients in China, therefore exerting predominant selective pressure for the emergence of resistance among pathogenic microorganisms. According to the report of China Nosocomial Pathogens Surveillance Study Group, the prevalence of ESBL-positivity among *E. coli* or *K. pneumoniae* isolated from ICUs in mainland China increased from 11% in 1994 to 34% in 2001 [24]. The higher percentage of ESBL-producing *E. coli* or *K. pneumoniae* in the current study may be due to the greater selective pressure imposed by extensive use of third-generation cephalosporins in NICU since floroquinolones and aminoglycosides were rarely used for pediatric patients because of concerns over their toxicity and lack of monitoring of drug concentration in this country. Indeed, our survey showed that the only antibiotic class that was significantly associated with the production of ESBL was third-generation cephalosporin. This finding is also in accordance with previous studies showing indiscriminate use of third-generation cephalosporins was related to the selection of ESBL-producing multi-resistant strains in NICU [6, 7]. These observations indicate that restriction of the extensive use of third-generation cephalosporins may help to decrease the acquisition of ESBL-producing *K. pneumoniae* in neonatal patients [25].

Ours results showed that ESBL-producing isolates were highly resistant to piperacillin, tobramycin, aztreonam, second- and third-generation cephalosporins, but remained susceptible to carbapenems, fluoroquinolones and beta-lactamase inhibitor combinations such as piperacillin–tazobactam, suggesting that these antimicrobial agents can be therapeutic options for ESBL-producing organisms. Nevertheless, the optimal therapy for infections caused by these pathogens has yet to be established. There were reports showing that imipenem had been the most successful antibiotics treating infections caused by ESBL-producing organisms and was superior to cephalosporins in for its rapid bacteriolysis with low levels of endotoxin release and stability to hydrolysis by ESBLS [26–29]. However, increased empirical use of carbapenems in response to ESBL-producing organisms has been accompanied by the rapid emergence of carbapenem resistance in *Pseudomonas aeruginosa* and other Gram-negative bacilli [30, 31].

Therapeutic options other than carbapenems for ESBL-producing organisms would be attractive. In the present study, the results of antimicrobial susceptibility tests suggested that beta-lactam-beta-lactamase inhibitor combinations such as piperacillin-tazobactam, and fluoroquinolones such as ciprofloxacin may constitute alternative antimicrobial therapy for ESBL-producing organism infection. Although ciprofloxacin has been found to cause irreversible injury to cartilage in juvenile laboratory animals [32], the literature review found that this complication occurs rarely in pediatric patients including premature infants [33]. Ciprofloxacin can be considered in the treatment of neonatal infection caused by multidrug-resistant Gram-negative organisms. However, data on ciprofloxacin treatment in neonatal patients are limited to case reports and it should only be reserved for the treatment of serious infections for which an alternative antibiotic is not available. Therefore, none of the case and control patients received ciprofloxacin treatment in this study.

It has been reported that multi-resistant bacteria may result in increased mortality among patients with nosocomial infection [34, 35]. However, in this study, only two patients in the case group died from ESBL-producing organism infection. The relative low mortality may be due to the fact that majority of case patients received appropriate antibiotic therapy, implying that appropriate empirical antimicrobial therapy may improve clinical outcome of patients with ESBL-producing organism infection.

The study by Piroth and associates [36] suggested that beta-lactamase inhibitors may have a protective effect against ESBL-producing *K. pneumoniae*, especially in intubated patients in ICU. However, in our study,

prior use of beta-lactamase inhibitors had no significant protection against ESBL-producing organisms. Unlike third-generation cephalosporins, beta-lactamase inhibitor may not increase the risk for ESBL-producing organism infection. One of the alarming finding of this study was that the resistance of ESBL-producing strains to amoxicillin–clavulanic acid, ticarcillin–clavulanic acid and cefoperazone–sulbactam was high. This is in consistence with reports that some clinical isolates of ESBL-producing *K. pneumoniae* were found to be less susceptible to beta-lactam-beta-lactamase inhibitor combinations because of hyperproduction of ESBLs [37, 38]. In addition, ESBL-producing *K. pneumoniae* can develop resistance to cefoxitin and expanded-spectrum-cephalosporins *in vitro* or *in vivo* by loss or decreased expression of porin channels for beta-lactamase inhibitor entry into the bacteria, indicating that other factors may interfere with susceptibility of bacteria to beta-lactamase inhibitor in addition to production of ESBLs [39]. Therefore, the role of beta-lactamase inhibitor therapy in severe neonatal nosocomial infection warrants further investigations.

There are several limitations in our study. One of which is that it is a clinical microbiological study rather than an epidemiological study because molecular epidemiologic analysis and characterization of ESBL types were not performed. Furthermore, the colonization rate of ESBL-positive bacteria was not available because of lack of routine screen for every infant admitted to the NICU. Our small sample size limits the statistical power to detect other possible risk factors for ESBL-producing organism infection. The lack of a strictly matched design between the case and the control groups makes it difficult to balance all confounding factors. By selecting as control patients those infected with non-ESBL producing bacteria, there is a possibility of introducing a systematic bias towards overestimation of the association with prior exposure to antimicrobial agents because patients receiving treatment which is active against non-ESBL producing bacteria are unlikely to become infected with such strains. A well-designed prospective trial balancing the confounding factors is needed to address this question properly.

In conclusion, preterm low birth weight, prolonged mechanical ventilation (≥ 7 days) and prior use of third-generation cephalosporins are risk factors for ESBL-producing *E. coli* or *K. pneumoniae* infection for neonatal patients in NICU. Interventions to reduce the duration of mechanical ventilation and avoid extensive use of third-generation cephalosporin may help to prevent ESBL-producing organism infection in neonates.

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