Emergence of high multidrug-resistant *Escherichia coli* isolates from neonates with infection

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Research

Abstract

Background *Escherichia coli* (*E.coli*) rank one of the most common pathogens that can cause neonatal infections. The emergence of antibiotic-resistant bacteria is a major cause of treatment failure in newborn with infection. The purpose of this study was to describe the antibiotic resistance and multidrug-resistance of *E.coli* isolated from neonates with infection.

Methods The antimicrobial susceptibility testing of the *E. coli* strains to selected antibiotics was assessed with the E-test technique on the Mueller-Hinton agar. The antimicrobials tests were included ceftazidime, cefuroxime, cefatriaxone, amoxicillin, amoxicillin-clavulanic acid, cefoperazone - sulbactam, meropenem, gentamicin, ciprofloxacin and sulfonamides. The minimal inhibitory concerntration (MIC) values of the antimicrobial agents selected for this study was determined by an agar dilution technique on Mueller-Hinton agar according to the Clinical and Laboratory Standards Institute recommendations.

Results A total of 100 *E. coli* strains was isolated from phlegm (n = 78), blood (n = 10), cerebrospinal fluid (n = 5), and umbilical discharge (n = 7) of neonates hospitalized at Beijing Children's Hospital. The highest resistance rate of *E.coli* was found in amoxicillin at 85%, followed by cefuroxime 65%, and cefatriaxone 60%, respectively. 6% and 5% of all isolates were resistant to amoxicillin/clavulanic acid and cefoperazone -sulbactam merely. The resistance rates to ceftazidime, gentamicin, ciprofloxacin and sulfonamides were 31%, 20%, 33%, 47%, respectively. All the isolates were susceptible to meropenem. Multidrug resistance was defined in *E.coli* as resistance to at least three antibiotic families. About 26% (26/100) of all the *E.coli* isolates were multidrug-resistant. The detection rate of ESBL-Producing *E. coli* was 55%. The rate in *E. coli* isolates from phlegm was higher than aseptic humoral. The difference was statistically significant ($P < 0.05$). It is worth noting that the majority of the isolates were also resistant to non-β-lactam antimicrobial agents, but the resistant rates were significantly lower than extended-spectrum β-lactamases.

Conclusions: Multi-drug-resistant *E.coli* has become a thorny problem in clinical treatment. It is necessary to monitor *E. coli* resistance.

Introduction

Newborns suffer high rates of mortality due to infectious disease[1]. Neonatal sepsis is the third leading cause of neonatal mortality, only behind prematurity and intrapartum-related complications (or birth asphyxia) [2].

Escherichia coli (*E. coli*) is the most common Gram-negative bacterium that can cause various diseases of both community and hospital acquired clinically significant blood stream infections (BSIs) and a major cause of mortality from these infections at all ages. According to infection site, pathogenic *E. coli* was divided into intestinal and extraintestinal *E. coli*, the latter category is called extraintestinal pathogenic *E. coli* (ExPEC). In recent years, many scholars in North America and Europe have reported ExPEC with serious pathogenicity in succession. A contemporary collection of 12737 strains from

pediatric patients (<18 years) isolated over a 7-year period (1998–2004) from 52 sentinel hospitals in North America showed that E. coli rank order of the top 6 pediatric pathogens [3]. ExPEC responsible for a leading cause of infections in neonate in gram-negative bacteria [4,5].

In the past few years, antibiotics have saved many lives and reduced the illness of many million people all over the world [3]. However, the remarkable benefits of antimicrobials in reducing morbidity and mortality have been challenged by the arise of drug resistant in recent years. For various reasons, the problem is more extruded in developing countries [2, 3]. The emergence and rapid spread of extended-spectrum cephalosporin and carbapenem resistance in Enterobacteriaceae is becoming a global health challenge. Antibiotic-resistant E.coli have been increasing and are becoming a major problem worldwide.

The emergence of multidrug-resistant E.coli, has been observed in various countries over the past decades. With the increase in cephalosporins resistance, especially the parallel increasing freguency of multidrug-resistant E.coli, concerns have been raised by experts with respect to the treatment of E.coli disease. The predominant mechanism of resistance to β-lactam antibiotics in E.coli is the production of plasmid-borne extended-spectrum β-lactamases (ESBLs). Since the first report at the beginning of the 1980s, ESBL-producing organisms have become widespread throughout the world [6]. The ESBL genes are frequently encoded on transferable plasmids that encode resistance genes. Acquisition of such resistant genes by commensal or fecal isolates leads to multidrug resistant (MDR) pathogens.

To the best of our knowledge, there are limited data regarding the antibiotic susceptibility of E.coli in neonatal invasive diseases worldwide, and especially in China. Therefore, The objective of the present study was to investigate the antibiotic susceptibility and multi-drug resistance of E.coli isolates that cause neonatal infections in order to provide a proper basis for clinical treatment of E.coli infections.

Materials And Methods

Study design

This study was carried on at the Beijing Children's Hospital, a tertiary hospital with 100 beds in the neonatal unit, which handles more than 3,000 inpatient neonates per year. Phlegm, blood and/or cerebrospinal fluid (CSF) samples were taken from inpatient neonates diagnosed with pneumoniae, sepsis and/or meningitis. Patients aged less than 28 days with E. coli-positive cultures were enrolled. This study was approved by the ethics committee of Beijing Children's Hospital, which was performed in accordance with the Declaration of Helsinki.

Bacterial identification

E. coli species identification was based upon ATB automatic bacterial identification instrument (France merrier company), VITEK automatic biological analysis system (Biomerier China company) or French merieres API system.

Antimicrobial susceptibility testing of *E.coli* and detection of ESBLs

The antimicrobial susceptibility testing of the *E. coli* strains to selected antibiotics was assessed with the E-test technique (AB Biodisk-solana, Sweden) on the Mueller-Hinton agar (Becton Dickinson). The antimicrobials tests were included ceftazidime, cefuroxime, cefatriaxone, amoxicillin, amoxicillin-clavulanic acid, cefoperazone - sulbactam, meropenem, gentamicin, ciprofloxacin and sulfonamides. The minimal inhibitory concerntration (MIC) values of the antimicrobial agents selected for this study was determined by an agar dilution technique on Mueller-Hinton agar (Oxoid) according to the Clinical and Laboratory Standards Institute (CLSIrecommendations[7]. *E. coli* ATCC 25922 was used for routine quality-control purposes. Multi-drug resistant (MDR) *E.coli* was defined as nonsusceptibility to at least one agent in three or more antimicrobial categories.

Detection of ESBLs

 The MICs of oxyimino-β-lactams and clavulanic acid were determined at a fixed concentration of 2mg/l. The production of *E. coli* Extended-spectrum beta-lactamases (ESBLs) was determined using the double-disk synergy test (DDST), which was performed with cefotaxime (30 μg) and ceftazidime (30 μg) disks placed at a distance of 20 mm (center to center) from the amoxicillin-clavulanic acid disk (20/10 μg). Moreover, cefpodoxime (10 μg) and aztreonam (30 μg) disks were added to increase the sensitivity of the DDST. Additionally, in the same culture medium, cefepime (30 μg) disk was placed, in order to improve the detection of ESBL when the simultaneous stable hyperproduction of an AmpC beta-lactamase occurs. The test result was considered positive when an enhancement of the inhibition zone around at least one of the antibiotic disks (cefotaxime, ceftazidime, cefpodoxime, aztreonam, or cefepime) toward the clavulanic acid disk was observed. Control strains Klebsiella pneumoniae ATCC 700603 (ESBL positive) and *E. coli* ATCC 25922 (ESBL negative) were used for quality control.

Statistical analysis

All data was set up and analyzed with the software WHONET 5.3 recommended by the WHO. The X^2 test was performed for comparing proportions using SPSS version 13.0 software. Differences with $P0.05$ were considered statistically significant.

Results

Characteristics of *E. coli* strains

A total of 100 *E. coli* strains was isolated from phlegm (n = 78), blood (n = 10), cerebrospinal fluid (n = 5), and umbilical discharge (n = 7) of neonates hospitalized at Beijing Children's Hospital.

Analysis of the antimicrobial susceptibility

The susceptibility to 10 antibiotics and the MICs of 100 *E.coli* isolates were presented in Table 1. Based on the CLSI 2016 criteria, the highest resistance rate of *E.coli* was found in amoxicillin at 85%, followed

by cefuroxime 65%, and cefatriaxone 60%, respectively. However, 6% and 5% of all isolates were resistant to amoxicillin/clavulanic acid and cefoperazone -sulbactam merely. The resistance rates to ceftazidime, gentamicin, ciprofloxacin and sulfonamides were 31%, 20%, 33%, 47%, respectively. All the isolates were susceptible to meropenem. The details of antimicrobial resistance rates were shown in Table 1.

Table 1 Susceptibility and MICs of 100 *E.coli* isolates to 10 antibiotics

Antibiotics	Susceptibility			MIC(μg/ml)		
	S^b(%)	I^b(%)	R^b(%)	50%	90%	Range
Ceftazidime	63	6	31	1.5	⬚256	0.016-256
Cefuroxime	35	0	65	⬚256	⬚256	0.016-256
Cefatriaxone	37	3	60	⬚32	⬚32	0.002-32
Amoxicillin	9	6	85	⬚256	⬚256	1.0-⬚256
Meropenem	100	0	0	0.016	0.094	0.002-32
Gentamicin	73	7	20	0.75	24	0.016-256
Ciprofloxacin	67	0	33	0.19	⬚32	0.002-32
Cefoperazone-sulbactam	77	18	5	3	16	0.016-256
Amoxicillin-clavulanic acid	72	22	6	3	24	1.5-16
Sulfonamides	53	0	47	0.125	⬚32	0.002-32

Multidrug-resistant *E.coli*

The antibiotic resistance pattern of 100 *E.coli* isolates was shown in Table 2. Amoxicillin, cefuroxime, cefatriaxone, ceftazidime, amoxicillin-clavulanic acid, cefoperazone - sulbactam and meropenem was classified as β-lactams, whereas gentamicin was classified as aminoglycoside. Furthermore, ciprofloxacin was regarded as quinolone. Multidrug resistance was defined in *E.coli* as resistance to at least three antibiotic families. About 26% (26/100) of all the *E.coli* isolates were multidrug-resistant.

Table 2 Antibiotic resistant pattern of 100 *E.coli* isolates.

Class of antibiotic	Resistance pattern	No. of isolates	Proportion of all isolates
0	-	5	5%
1	β-lactams	26	26%
2	β-lactams+ aminoglycoside	5	5%
	β-lactams+ quinolon	19	19%
	β-lactams+ sulfonamides	19	19%
3	β-lactams+ aminoglycoside+quinolon	4	4%
	β-lactams+ aminoglycoside+sulfonamides	3	3%
	β-lactams+quinolon+sulfonamides	16	16%
4	β-lactams aminoglycoside+quinolon+sulfonamides	3	3%

Inspection situation of ESBLs

The detection rate of ESBL-Producing *E. coli* was 55%. The rate in *E. coli* isolates from phlegm (65%, 51/78) was higher than aseptic humoral (27%, 4/15). The difference was statistically significant ($P < 0.05$).

Susceptibility of ESBL-Producing *E.coli* to Antimicrobial Agents

It is worth noting that the majority of the isolates were also resistant to non-β-lactam antimicrobial agents, but the resistant rates were significantly lower than extended-spectrum β-lactamases. The results for cefuroxime, cefatriaxone, amoxicillin were significant with $P = 0$, $P = 0$, $P = 0.017$. The results for ceftazidime, gentamicin, ciprofloxacin, cefoperazone-sulbactam, amoxicillin-clavulanic acid, sulfonamides were insignificant with $P = 0.1998$, $P = 0.417$, $P = 0.764$, $P = 0.8$, $P = 0.5$, $P = 0.354$, respectively, shown in Table 3.

Table 3 Susceptibility profile of ESBL-Producing and non ESBL-Producing *E.coli* strains

	ESBL	Non ESBL	X^2	P
Ceftazidime	20(36.4)	11(24.4)	1.644	0.1998
Cefuroxime	51(92.7)	14(31.1)	41.303	0.00
Cefatriaxone	47(85.5)	13(28.9)	32.997	0..00
Amoxicillin	51(92.7)	34(75.6)	5.724	0.017
Meropenem	0	0	-	-
Gentamicin	12(21.8)	13(28.9)	0.66	0.417
Ciprofloxacin	19(34.5)	14(31.1)	0.09	0.764
Cefoperazone-sulbactam	3(5.5)	3(6.7)	0.064	0.8
Amoxicillin-clavulanic acid	5(9.1)	6(13.3)	0.455	0.5
Sulfonamides	23(41.8)	23(51.1)	0.86	0.354

Discussion

Escherichia coli is the most frequent Gram-negative organism that causes neonatal bacteremia and sepsis[8]. Among febrile infants≤28 days-old, the prevalence of bacteremia and meningitis is high, *E.coli* is one of the most common bacterial pathogens[9]. The incidence of *E. coli* early-onset sepsis in very low birth weight infants was 10.4 cases per 1000 live births and mortality reached 35.3%[10]. A recent meta-analysis based on a systematic review of the published studies in Chinese literature demonstrates that in newborn infants hospitalized in Chinese NICUs, roughly 50% of all *E. coli* bloodstream isolates (regardless of early onset or late onset) are multi-drug resistant due to extended-spectrum beta-lactamase (ESBL) production[11-12].

Emerging antibiotic resistance is currently acknowledged as one of the most significant public health problems and mortality rates since multidrug-resistant bacterial infections are high. The selective pressure of antimicrobial use, overuse and misuse comprises the engine driving this process leading to a gradual increase in antibiotic resistance. Subsequently, once treatable bacteria are now either untreatable or require the last line of antibiotics [13]. Among the resistant bacteria, *E.coli* is the most common Gram-negative bacterial pathogen, causing a diverse range to clinical diseases that affect all age groups. Multidrug-resistant, extensively drug-resistant and pan-drug-resistant strains of *E.coli* is now reported worldwide, becoming a critical global issue[14].

The current commonly used clinically to treat gram-negative bacilli infection mainly cephalosporins, belongs to β-lactam antibiotics. Producing extended spectrum beta lactamase (ESBL) is the main mechanism of *E.coli* resistant to beta lactam antibiotics. ESBL is a plasmid-mediated β-lactamase that is capable of hydrolysing and inactivating β-lactams such as cephalosporins and monobactams[15]. Identification of ESBL-producing *E.coli* (ESBL-*E.coli*) infections in infants in a neonatal intensive care unit is of particular concern because of the hosts' immature antibacterial immunity and restricted therapeutic antibiotic options [16].

The *E.coli* isolates studied displayed resistance patterns typical of ESBL producers. In our study, the majority of the isolates studied were resistant to amoxicillin (MIC range: 1.0 to ⬜256mg/l), but a very few *E.coli* were resistant to amoxicillin in combination with clavulanic acid (MIC range: 1.5-16). The antimicrobial resistance of experimental strains showed great difference to amoxicillin and amoxicillin-clavulanic acid, in other words, they can hydrolyzes cephalosporins, but this hydrolysis can be inhibited by clavulanic acid. According to Karen's research[17], these clinical *E.coli* isolates may produce group 2e β-lactamases. In Beltran study, *E.coli* strains isolated from urine cultures of patients from Primary Care Barbastro Sector, between January 2011 and December 2013, only amoxicillin-clavulanate had increased progressively reaching 21.5% in 2013, and presented a statistically significant increase[18], which was significantly higher than that of our study. *E.coli* isolated from neonatal unit showed a high resistance to amoxicillin, which was consistent with the results of Nitsch-Osuch [19]. They described a relatively low degree of resistance to cephalosporins (1.8-5.3%) and aminoglycosides (0-2.6%), which was lower than our study. Bergin[20] used multivariable logistic regression to evaluate the association between 30-day mortality and ampicillin-resistant *E.coli* bloodstream infections, and identified 123 (48%) ampicillin-resistant isolates. They found that ampicillin resistance was not associated with significantly increased mortality, and appropriate empirical antibiotic therapy was not associated with lower mortality.

Monsef [21]reported a higher resistance of *E.coli* cultured from neonatal patients to cephalosporins and aminoglycosides. In our study, the most isolates of *E.coli* were resistant to cefuroxime (65 out of 100, MIC range: 0.016-256μg/ml), cefatriaxone (60 out of 100, MIC range: 0.002-32μg/ml). Moreover, all the isolates were susceptible to meropenem (MIC:⬜0.002μg/ml), and the vast majority strains were susceptible to cefoperazone in combination with clavulanic acid (MIC range: <0.016 to 32μg/ml). It is worth noting that the majority of the *E.coli* strains were also resistant to non-β-lactam antimicrobial

agents. A part of *E.coli* strains were resistant to sulfonamides (47 out of 100, MIC range: 0.002-32μg/ml), ciprofloxacin (33 out of 100, MIC range: 0.002-32μg/ml), gentamicin (20 out of 100, MIC range: 0.016-256μg/ml), respectively. As previously reported [22], tigecycline demonstrates excellent activity against a wide variety of Gram-positive and Gram-negative bacteria, including ESBL-producing organisms, which could be considered as an encouraging antimicrobial. However, this antibiotic is not recommended in adolescents under 18 years of age due to lack data on its safety, let alone neonates [6].

Vernaz [23] performed a retrospective observational time-series analysis to evaluate the incidence of non-duplicate clinical isolates of *E.coli* resistant to ciprofloxacin, trimethoprim/sulfamethoxazole and cefepime from January 2000 through December 2007, they observed an increase in fluoroquinolone resistance among CA and HA isolates of *E.coli*, with slightly higher rates in the latter group, in line with data from most other European countries. They noted that the rate of ciprofloxacin resistance in *E.coli* is approaching the resistance rate of trimethoprim/sulfamethoxazole, they found that ciprofloxacin and cefepime resistance both increased, Trimethoprim/sulfamethoxazole resistance remained stable, while total antibiotic use increased in both inpatient and outpatient settings. The results support efforts to reduce prescribing of fluoroquinolones for control of resistant *E.coli* including extended-spectrum β-lactamase producers.

In our study, we took meropenem as treatment option to multidrug-resistant *E.coli* bacteremia. The recommended dosage of meropenem is calculated with 20mg/kg q8h in neonate. When it was used in neonates, more concerns should be taken because of possible side effects such as anaphylaxis, liver and kidney impairment and haemorrhage symptom. Although all the *E.coli* isolates were sensitive to meropenem in our study, it is hard to evaluate the efficacy and the safety of carbapenems in pediatric patients, especially in neonates. More concerns should be taken because of its possible side effects. Meropenem and Imipenem are both members of carbapenems, a clinically important antibiotic family used in the treatment of Multidrug-Resistant (MDR) bacterial infections. However, susceptibility tests performed by the Kirby-Bauer disk diffusion method demonstrate that Imipenem sensitive *E.coli* BL21 cells overexpressing Ar-BVMO become resistant to this antibiotic. Agar disc diffusion assay proves that when Imipenem reacts with Ar-BVMO, it loses its antibiotic property [24].

More than quarter of the isolates were resistant to at least three different classes of antibiotics in our study. A notable proportion to cephalosporin resistant probably reflects the epidemiology of ESBL-producing Enterobacteriaceae in our region, as in the rest of Asia.

Conclusion

Drug-resistant *E.coli* has become a thorny problem in clinical treatment. Additional up-to-date information about *E. coli* resistance among newborn will help to inform clinical evaluation and decision-making. It is necessary to monitor *E. coli* resistance.

Abbreviations

E.coli:Escherichia coli; ESBL:Extended-spectrum beta-lactamase; NICU:Neonatal intensive care unit; ExPEC:Extraintestinal pathogenic *E. coli;* CSF:Cerebrospinal fluid

Declarations

Acknowledgments

Not applicable.

Authors' contributions

YJW designed the study. DW collected the data. YJD, JJZ, KHY, WG, YL and XJW were participants in the workshop and the round-table and either gave presentations. DW analyzed the data and wrote the first draft of the manuscript, which was significantly edited by YJW. All authors read and approved the final manuscript.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

A parent and/or legal guardian of each participant signed a written informed consent document before enrollment and before any study procedure was performed. This study was viewed and approved by the Ethics Committee of Beijing Children's Hospital Affiliated to Capital Medical University. No ethical problems existed in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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