Analysis of drug resistance of extended-spectrum beta-lactamases-producing *Escherichia coli* and *Klebsiella pneumoniae* in children with urinary tract infection

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ABSTRACT

الأهداف: دراسة مقاومة العقاقير لبيتا لاكتاماز بيتا لاكتاماز (ESBL) المنتجة للطفيليات الإشريكية القولونية (*E. coli*) والكلبسيلة الرئوية (*K. pneumoniae*) في الأطفال المصابين بعدوى المسالك البولية (UTI) وتوفير الأساس المنطقي للاستخدام السريري للمضادات الحيوية.

المنهجية: أجري تحليل بأثر رجعي لمدى حساسية الدواء لدى الأطفال المصابين بالإشريكية القولونية أو الكلبسيلة الرئوية وكانت مزرعة البول إيجابيه خلال الفترة من أغسطس 2013م وأغسطس 2017م، مستشفى شنتشن للأطفال ، شنتشن ، الصين. تم تقييم المقاومة الدوائية إحصائيا باستخدام اختبار فيشر الدقيق واختبار كاي.

النتائج: أثبتنا 698 حالة من الإشريكية القولونية ، تم تأكيد وجود 426 منها للإصابة بسلالات ESBL، و 217 حالة من حالات الكلبسيلة الرئوية بما في ذلك 111 سلالة ESBL المنتجة ، تم الكشف ، والفرق في نسبة سلالات ESBL الإيجابية المنتجة 61.03% مقابل 61.03% كانت ذات دلالة إحصائية. كانت السلالات (61.03% مقابل 51.15%) ذات دلالة إحصائية (p=0.010). كان متوسط معدلات مقاومة العقاقير من الإشريكية القولونية والالتهاب الرئوي للبيبيراسيلين / تازوباكتام ، الميروبينيم ، الإرتابينيم ، الإيميبينيم ، والأميكاسين أقل من 15%. كان متوسط معدلات المقاومة للبكتيريا القولونية المنتجة للESBL و الإشريكية القولونية للسيفبودوكسيم و السيفيكسيم وسيفازولين و سيفترياكسون أكثر من 98%، بينما كان متوسط معدلات المقاومة للبكتيريا غير المنتجة للESBL للأدوية 4 المذكورة أعلاه أقل من20%.

الخلاصة: في جنوب الصين ، كانت نسبة السلالات المنتجة للESBL ومعدلات مقاومة العقاقيرلإشريكية القولونية والكلبسيلة الرئوية عند الأطفال مرتفعة، لكن معدلات مقاومتهم لمركبات الكاربابينيمات ومثبطات β–lactamase المحتوية علىtazobactam كانت منخفضة. تعد الكاربابينيمات من الأدوية الأكثر فعالية للجراثيم لعلاج البكتيريا المنتجة للESBL.

Objectives: To investigate the drug resistance of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) in children with urinary tract infection (UTI) and to provide the rationale for clinical use of antibiotics.

Methods: This is a retrospective analysis of drug susceptibility in children with *E. coli* or *K. pneumoniae*-positive urine culture between August 2013 and August 2017, Shenzhen Children's Hospital, Shenzhen, China. Drug resistance was statistically assessed using Fisher exact test and χ^2 test.

Results: A total of 698 cases of *E. coli*, 426 of which were confirmed ESBL-producing strains, and 217 cases of *K. pneumoniae*, including 111 ESBL-producing strains, were detected, and the difference in proportion of positive ESBL-producing strains (61.03% versus 51.15%) was statistically significant (p=0.010). The average drug resistance rates of *E. coli* and *K. pneumoniae* to piperacillin/tazobactam, meropenem, ertapenem, imipenem, and amikacin were <15%. The average resistance rates of ESBL-producing *E. coli* and *K. pneumoniae* to cefpodoxime, cefixime, cefazolin, and ceftriaxone was >98%, while average resistance rates for non-ESBL-producing bacteria to the above 4 drugs was <20%.

Conclusion: In southern China, the proportion of ESBL-producing strains and the drug resistance rates of *E. coli* and *K. pneumoniae* in UTI in children was high, but their resistance rates to carbapenems and β-lactamase inhibitor complexes containing tazobactam were low. Carbapenems are the most effective antibacterial drugs for the treatment of ESBL-producing bacteria.

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Urinary tract infection (UTI) is a common infectious disease in children, and 5.6% to 10.2% of children will subsequently develop permanent renal scars,[1] leading to serious complications such as renal dysplasia, recurrent pyelonephritis, impaired glomerular function, early hypertension, and end-stage renal disease.[2] Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli (E. coli)* and *Klebsiella pneumoniae (K. pneumoniae)* are important challenges in the treatment of UTIs in children due to their high incidence and multidrug resistance. There are significant regional differences in the types and detection rates of ESBL-producing bacteria. Studies have shown a gradual increase in the number of ESBL-producing strains isolated from *E. coli* or *K. pneumoniae*.[3] Understanding epidemic UTI pathogens and their drug resistance characteristics will improve clinical efficacy and reduce the emergence of drug-resistant strains through empirical drug use. Large numbers of ESBL-producing strains have not been reported in children with UTIs in southern China.

This study intends to analyze the drug resistance of *E. coli* and *K. pneumoniae*, isolated from urine specimens of hospitalized children with UTIs in Shenzhen Children's Hospital, Shenzhen, China thereby providing information on the drug resistance of ESBL-producing bacteria.

Methods. The drug susceptibility results of hospitalized children with UTIs admitted to Shenzhen Children's Hospital between August 2013 and August 2017 were collected. The study included 576 males and 339 females with *E. coli*- or *K. pneumoniae*-positive urine cultures with colony number >10^5/ml. The ratio of males to females was 1.7:1. The age range of the patients was 11 days to 14 years with a median age of 12 months. Patients were excluded if their infections were caused by different pathogens within 2 weeks after anti-infective treatment, or the same pathogen within 4 weeks after anti-infection treatment.

For children who had undergone toilet training, mid-stream urine was collected after cleaning their vulva and urethra. For children with indwelling catheters and non-toilet-trained young children, the catheterization method was applied. Urine specimens were sent for inspection in sterile containers within half an hour of collection. Specimen cultures were performed according to the requirements of the National Clinical Laboratory Procedures. Strain identification and drug susceptibility tests were carried out by VITEK®2 compact 60 automatic microbial identification and drug sensitivity analyzer (BioMerieux, France). On the basis of the Clinical and Laboratory Standards Institute (CLSI) guidelines, drug resistance, intermediate sensitivity and susceptibility were determined. The minimum inhibitory concentration values were detected by the microdilution method. The standard quality control strains were *E. coli* ATCC 25922 and *K. pneumonia* ATCC 700603 (National Center for Clinical Laboratories, Beijing, China). This study has passed the ethical review of the Ethics Committee of Shenzhen Children's Hospital.

Statistical analysis. The data were analyzed by the Statistics Package for Social Sciences for Windows, version 25.0 (IBM Corp, Armonk, NY, USA). The comparison between the data groups was tested by χ^2, if the effective value is <5, the Fisher exact test was adopted. A p value <0.05 was considered statistically significant.

Results. A total of 698 cases of *E. coli,* 426 of which were ESBL-producing, and 217 cases of *K. pneumoniae* including 111 cases of ESBL-producing strains were detected in this study. The overall prevalence of ESBL-producing strains was 58.69%. The prevalence of ESBL-producing *E. coli* was higher than that of *K. pneumonia* (61.03% versus 51.15%), and the difference in prevalence between them was statistically significant (χ^2=6.664, p=0.010).

Sensitivity testing of culture strains to antibiotics commonly used in clinical practice showed that the resistance rates of *E. coli* and *K. pneumoniae* to piperacillin/tazobactam, meropenem, ertapenem, imipenem, and amikacin were <15%.

The resistance rates of ESBL-producing *E. coli* and *K. pneumoniae* to cefpodoxime, cefaclor, cefazolin, and ceftriaxone were >98%, whereas the resistance rates of non-ESBL-producing bacteria to the above 4 drugs were <20%. The difference was statistically significant (p<0.05).

The resistance rates of *E. coli* to nitrofurantoin was 7.61% and for *K. pneumoniae* was 72.22%, and the difference was statistically significant (χ^2=3.634×10^2, p=0.000, **Table 1**).

Discussion. Since ESBL-producing *K. pneumoniae* was first discovered in the 1980s, it has spread worldwide. There are significant differences in the types and detection rates of ESBL-producing bacteria

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Table 1 - Comparison of drug resistance between *Escherichia coli* and *Klebsiella pneumoniae* in extended-spectrum beta-lactamase-producing group and non-extended-spectrum beta-lactamase-producing group (%).

Antibacterial drugs	*Escherichia coli*					*Klebsiella pneumoniae*				
	ESBLs (+)		ESBLs (-)			ESBLs (+)		ESBLs (-)		
	Number of resistant strains	Resistance rate	Number of resistant strains	Resistance rate	*P*-value	Number of resistant strains	Resistance rate	Number of resistant strains	Resistance rate	*P*-value
Ampicillin/sulbactam	366	86.94	132	55.93	0.000	109	99.09	27	30.68	0.000
Piperacillin/tazobactam	7	1.66	3	1.27	1.000	16	14.55	10	11.36	0.510
Cefpodoxime	402	100.00	7	4.05	0.000	103	100.00	14	16.47	0.000
Cefixime	255	100.00	3	2.07	0.000	69	100.00	6	11.11	0.000
Meropenem	1	0.25	1	0.44	1.000	1	0.98	5	6.25	0.088
Ertapenem	5	1.19	1	0.43	0.428	2	1.87	7	8.54	0.738
Aztreonam	228	54.29	3	1.27	0.000	81	73.64	11	12.50	0.000
Amikacin	7	1.66	2	0.85	0.501	2	1.82	1	1.14	1.000
Levofloxacin	157	37.20	38	16.10	0.000	12	10.91	9	10.23	0.877
Nitrofurantoin	42	9.95	8	3.40	0.002	82	74.55	61	69.32	0.414
Cefazolin	420	99.76	27	11.44	0.000	110	100.00	16	18.18	0.000
Cefaclor	406	100.00	15	15.31	0.000	103	100.00	13	37.14	0.000
Ceftazidime	141	33.41	5	2.12	0.000	69	62.73	14	15.91	0.000
Ceftriaxone	420	99.53	13	5.51	0.000	108	98.18	15	17.05	0.000
Cefepime	138	32.70	2	0.85	0.000	55	50.00	10	11.49	0.000
Imipenem	1	0.24	1	0.42	1.000	6	5.45	10	11.36	0.130
Cefotetan	6	1.42	2	0.85	0.718	5	4.55	12	13.64	0.023
Tobramycin	179	42.42	73	31.06	0.004	42	38.18	10	11.36	0.000
Gentamicin	174	41.23	75	32.05	0.020	38	34.55	9	10.23	0.000
Ciprofloxacin	175	41.47	38	16.10	0.000	21	19.09	10	11.49	0.146
Sulfamethoxazole	272	64.45	102	43.40	0.000	61	55.45	18	20.45	0.000

ESBLs - extended-spectrum beta-lactamases

in various countries and regions. *Escherichia coli* is the main pathogen causing UTI; *E. coli* can invade bladder epithelial cells, escape into the cytoplasm of host cells and form intracellular bacterial communities, allowing bacterial replication and avoiding host immune responses.[4] *Escherichia coli* and *K. pneumoniae* are the most common ESBL-producing bacteria; indwelling catheterization, history of recurrent urinary tract infection and use of antibiotics within 3 months are risk factors for ESBL production.[5] The detection rates are higher in Latin America, Asia, and the Middle East but lower in the South Pacific, Europe, and North America,[6,7] considering the abuse and non-standard use of antibiotics in developing countries. It has been reported that from 2000 to 2009, the detection rate of ESBL-producing *E.coli* increased from 3.3% to 8% and that of *K. pneumoniae* increased from 9.1% to 18.6%.[8] The overall rate of UTIs, caused by ESBL-producing bacteria, in our hospital was 58.69%, and the proportions positive for ESBLs-producing was 61.03% *E. coli* and 51.15% *K. pneumoniae.* These data indicate that the region has a high detection rate of ESBL-producing bacteria. Extended-spectrum beta-lactamases are plasmid-mediated, widely active β-lactamases that hydrolyze penicillins and cephalosporins by cleavage of the amide bond of the β-lactam ring to inactivate β-lactam antibiotics; therefore, inferring resistance to first to 3rd generation cephalosporins and aztreonan antibiotics.[9] At the same time, these plasmids usually carry additional resistance genes to other drugs, such as aminoglycoside, sulfonamides, and quinolones, to make the bacteria multidrug-resistant,[10] which often leads to serious infections. By understanding the epidemiological and drug resistance characteristics of ESBL-producing bacteria, antibiotics can be used in a targeted manner to avoid inappropriate antibacterial treatment and the increase of resistant strains producing ESBLs.

In this study, ESBL-producing *E. coli* and *K. pneumoniae* were widely resistant to cephalosporins. In addition to cefotetan, resistance to cefepime, a 4th generation cephalosporin, was evident in 32.7% of cases. Thus, we recommend that unless there is a clear indication of drug susceptibility, cephalosporin should be cautiously used for treatment of UTIs in children with ESBL-producing bacteria. The activity of ESBL-

producing bacteria can be inhibited by β-lactamase inhibitors such as sulbactam, clavulanic acid, and tazobactam. The currently recommended antibacterial agents for ESBL-producing bacteria are combination preparations of carbapenems and β-lactamase inhibitors. It is also possible to use aminoglycosides and fluoroquinolones in combination with the above drugs based on drug susceptibility results and conditions. Carbapenems are considered first-line treatment for ESBL infections.[11] Extended-spectrum beta-lactamase-producing bacteria are sensitive to different combination preparations, such as cefoperazone/sulbactam and piperacillin/tazobactam, but resistant to amoxicillin/clavulanic acid and ampicillin/sulbactam. This is consistent with the results of our study, which showed low resistance rates of ESBL-producing (1.66% *E. coli* and (14.55%) *K. pneumoniae)* to piperacillin/tazobactam and high resistance rates to ampicillin/sulbactam (86.94% *E. coli* and 99.09% *K. pneumoniae*). A combination of piperacillin/tazobactam is preferred for mild to moderate infections caused by ESBL-producing bacteria, whereas carbapenems may be selected for severe infections. While other antibacterial agents have poor efficacy, carbapenem antibiotics act quickly and stably and can resist the hydrolysis of ESBLs, as supported by sufficient clinical data. Therefore, carbapenems are currently the most effective antibacterial agents for the treatment of ESBL-producing bacteria.[11-13] The resistance rate to meropenem, ertapenem, and imipenem in this group was 0.24%-5.45%, which confirmed the high sensitivity of carbapenem antibiotics to ESBLs-producing bacteria. It is worth noting that 3 carbapenem antibiotics that produce ESBLs from *E. coli* and *K. pneumoniae* in *in vitro* susceptibility tests have been found in the drug-resistant strains. Therefore, it is necessary to strictly control the indications for drug use in clinical treatment. Further increase of drug-resistant strains can be avoided by adequate medium dose, appropriate course of treatment, and alternating use of antibacterial drugs.

Although ESBL-producing bacteria carry aminoglycoside-resistant genes, our study found that ESBL-producing bacteria have a resistance rate of <2% to amikacin, which is significantly lower than that of gentamicin and tobramycin. However, these drugs have ear and kidney toxicity in children and need to be used with caution. Therefore, they are only used as a combination therapy for patients with severe infections caused by ESBL-producing bacteria. Ciprofloxacin and levofloxacin affect cartilage development in infants and young children, and their sensitivity rate in our study has no obvious advantage over aminoglycoside and β-lactamase inhibitor complex preparations, which is consistent with the resistance of fluoroquinolones to gram-negative bacilli causing UTIs found by Hoban et al.[14] Hence, we do not recommend the use of fluoroquinolone antibiotics for the treatment of UTIs in children.

Some studies have shown that nitrofurantoin has better kidney metabolism and is recommended for the treatment of complex UTIs.[15] However, there are also studies suggesting that nitrofurantoin is only used as a preventive drug and not for the treatment of complicated UTIs.[16] The American Urological Association 2010 guidelines consider that there is no theoretical basis for continuous prophylactic use of antibiotics. It is recommended that infants with only vesicoureteral refluxand those with bladder and bowel dysfunction have prophylactic antibiotics. In our study, the resistance rates of *E. coli* and *K. pneumoniae* to nitrofurantoin were significantly different (7.61% versus 72.22%). We recommend nitrofurantoin as a preventive medication for the treatment of UTIs caused by *E. coli* and recurrent UTIs. The gastrointestinal side effects of nitrofurantoin in children need to be considered while using the drug.

Study limitation. It is only a single-center study, and the time span is not long enough to reflect the trend of ESBL- producing bacteria; multi-center, longer-term research would be more reflective of the real situation. It is necessary to strictly control the indication for medication and adjust the antibacterial drugs over time according to the results of drug susceptibility tests. The use of antibiotics in sufficient amounts and over sufficient courses of treatment can reduce the production of resistant bacteria producing ESBLs and improve the clinical treatment effect.

This study will advise clinicians in the region on the clinical use of drugs, it will simultaneously inform other regions on the situation of ESBL-producing bacteria in South China.

In conclusion, the proportion of UTIs in children caused by ESBL-producing bacteria is high in South China, and the drug resistance is serious. For the mild to moderate UTIs caused by ESBL-producing *E. coli* and *K. pneumoniae*, the β-lactamase inhibitor complex containing tazobactam should be preferred. Carbapenems can be selected for patients with severe or poor efficacy. Nitrofurantoin can be used to prevent UTIs caused by *E. coli*.

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