Macedonian Journal of Medical Sciences. 2009 Mar 15; 2(1):36-41.
doi:10.3889/MJMS.1857-5773.2009.0030
Basic Science

OPEN ACCESS

Extended-Spectrum Beta-Lactamase-Producing *E. Coli* and *Klebsiella Pneumoniae* in Children at University Pediatric Clinic in Skopje

Ana Kaftandzhieva, Vesna Kotevska, Gordana Jankoska, Biljana Kjurcik-Trajkovska, Zhaklina Cekovska, Milena Petrovska

Institute of Microbiology and Parasitology, Medical Faculty, University "Ss Kiril and Metodij", Skopje, Republic of Macedonia

Key words:
Extended-spectrum β-lactamase producing *Escherichia coli*; ESBL-producing *Klebsiella pneumoniae*; antibiotic susceptibility.

Correspondence:
Kaftandzhieva Ana
Institute of Microbiology and Parasitology, Medical Faculty, University "Ss Kiril and Metodij", Skopje, Republic of Macedonia
Tel.: 389 2 3109829
Fax: 389 2 3214317
E-mail: akaftandzieva@yahoo.com

Received: 01-Dec-2008
Accepted: 13-Feb-2009
Online first: 16-Feb-2009

Abstract

Aim. The objectives of this study were to determine the prevalence and antibiotic susceptibility patterns of extended-spectrum β-lactamases (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae.*

Material and methods. *E. coli* and *K. pneumoniae* were obtained from all clinical samples of hospitalized children.

Results. During one year period, 212 strains of *E. coli* and 103 strains of *K. pneumoniae* were isolated. Of these, the ESBL production was observed in 26 (11.8%) isolates of *E. coli* and 25 (24.3%) isolates of *K. pneumoniae*. ESBL-producing *E. coli* isolates were commonly recovered from the respiratory tract (21.4%) and urine (7.2%). ESBL-positive *K. pneumoniae* was commonly recovered from urine (38.5%) and respiratory tract (18.7%). ESBL-positive *E. coli* isolates were more susceptible to AMC (76%) and SXT (50%), than were the isolates of ESBL-positive *K. pneumoniae* (40% and 32%). Considering aminoglycosides, 92% of ESBL-positive *E. coli* and 60% of ESBL-positive *K. pneumoniae* were susceptible to amikacin vs. 23% and 40% of ESBL-positive *E. coli* and *K. pneumoniae* strains to gentamicin. ESBL-positive *K. pneumoniae* strains were more susceptible to ciprofloxacin (84%) than ESBL-positive strains of *E. coli* (38%). Cefepime shows the best in vitro activity of tested cephalosporins (58% for *E. coli* and 72% for *K. pneumoniae)*. All isolates were susceptible to imipenem.

Conclusion. ESBL-producing *E. coli* and *K. pneumoniae* are present in our hospital environment. It's necessary and useful to perform screening and confirmatory tests for phenotypic detection of those organisms in a routine work. Most of ESBL-producers are resistant to many classes of antibiotic, resulting in limited treatment options.

Introduction

Bacterial antibiotic resistance has become a major clinical concern worldwide. Recently, the use of second and third generation cephalosporins has led to the selection of Gram-negative organisms resistant to β-lactamase stabile cephalosporins. This resistance is attributed to the production of extended-spectrum β-lactamases (ESBL). These enzymes are plasmid-mediated and they confer resistance to oxyimino-

cephalosporins (cefotaxime, ceftriaxone, ceftazidime etc) and to monobactams (aztreonam), but they are not active against cephamycins and carbapenems. ESBLs are most commonly found in *Klebsiella spp* and *Escherichia coli*, but they have also been detected in other members of the *Enterobacteriaceae* family. Currently more than 150 different types of ESBLs have been identified. The emergence of such strains has important clinical and therapeutic implications. First, many of these enzymes are often derived from TEM and SHV enzymes, which are present in 75% of *Enterobacteriaceae*. The resistance determinants for ESBLs are often found on transmissible plasmids, which facilitate the spread of the determinants among members of the *Enterobacteriaceae* family. Because of multiresistant plasmids that may be easily transmitted, ESBL producing organisms are often resistant to other classes of antibiotics. Hence, a most appropriate name would be "multidrug resistant organisms". Second, because of the lack of an obvious marker to indicate the presence of such enzymes, routine susceptibility testing may not detect the presence of ESBLs (1,2).

Since the first isolation of ESBL production by *Klebsiella pneumoniae* strain in 1983 and *E. coli* strain in 1987, a numerous outbreaks caused by these organisms have been reported worldwide. Most outbreaks have occurred in intensive care units (ICUs), as well as in other areas of the hospital such as surgical wards, pediatric and geriatric wards. Risk factor for acquisition of ESBL-producing organisms include length of hospital or ICU stay, severity of illness, intravascular or urinary catheterization, low birth weight, previous exposure to antibiotics etc. (3-6).

There have been many reports of infection caused by ESBL-producing *E. co*li and *Klebsiella pneumoniae.* However, limited information is available on these infections in children. Thus, the objectives of this study were to determine the prevalence and antibiotic susceptibility patterns of ESBL-producing *E. coli* and *K. pneumoniae* as a cause of infection or colonization of children hospitalized at University Pediatric Clinic in Skopje.

Material and methods

Bacterial isolates

E. coli and *Klebsiella pneumoniae* were obtain from all clinical samples (tracheal aspirates, urine, blood for hemoculture, wounds and others) of children hospitalized at Pediatric clinic in a one year period (January-December 2007). Only one strain from each patient was included in this study. All isolates were identified by standard microbiological techniques.

Susceptibility testing

Antimicrobial susceptibility testing of all isolates was performed by disk diffusion method. The inoculum was adjusted to the turbidity of a 0.5 McFarland standard and swabbed onto the surface of a Muller-Hinton agar plate. After putting the disks onto the inoculated plates, the plates were incubated at 37^0C for 24 hours. All disks were obtained from Oxoid Ltd., UK. Antibiotic potency of the discs was standardized against the reference strain *E. coli* ATCC 25922. All susceptibility results were interpreted according to the CLSI (Clinical and Laboratory Standards Institute). The following antimicrobial agents were used for susceptibility testing: cefixime (CFM), cefrtiaxone (CRO), cefuroxime (CXM), cefepime (FEP), piperacillin (PIP), amoxicillin-clavulanic acid (AMC), imipenem (IMI), gentamicin (G), amikacin (AK), ofloxacin (OFL), ciprofloxacin (CIP) and ko-trimoxazole (SXT), pipemidic acid (PI), nitrofurantoin (F), ofloxacin (OFL) and norfloxacin (NOR).

Detection of ESBL

For detection of ESBL- production, modify double disk test was performed as a screening test. ESBL-production and susceptibility to antimicrobial agents can be detected on the same plate. This test is suitable for routine work. Susceptibility testing was performed as previously described. Disk containing clavulanic acid (AMC) was placed between at least two cephalosporin disks in a distance of 30 mm (centre to centre). The enhancement of the zone of inhibition of any of disks towards the disk containing clavulanic acid suggested the presence of an extended-spectrum beta-lactamase.

ESBL set (Mast Diagnostic) is commercially available and it was used as confirmatory test for phenotypic detection of ESBL production. ESBL set contains 3 paired sets of cephalosporins (ceftazidime-CAZ; cefotaxime-CTX and cefpodoxime-CPD) and cephalosporins with clavulanic acid-CA (CAZ+CA; CTX+CA and CPD+CA). These pairs of disks were placed onto the inoculated agar plates. The zones of inhibition for the CAZ, CTX and CPD to that of the same cephalosporins plus clavulanic acid combination disks were compared. An increase in zone diameter of e" 5 mm in the presence of clavulanic acid from any or all of the sets of ESBL Detection disks, indicates the presence of ESBL in the test organisms.

Results

During the study period a total of 212 isolates of *E.coli* and 103 isolates of *Klebsiella pneumoniae* were obtained from clinical samples. Of these, the ESBL production was observed in 26 (11.8%) isolates of *E. coli* and 25 (24.3%) isolates of *K. pneumoniae*. Table 1 shows the number of ESBL-producing *E. coli* and *K. pneumoniae* isolates from various types of clinical samples.

Table 1: Clinical samples of children infected or colonized with ESBL-producing *E. coli* and *Klebsiella pneumoniae* isolates.

Specimen	Total number of *E. coli*	*E. coli* - ESBL +	Total number of *K. pneumoniae*	*K. pneumoniae* - ESBL+
Tracheal aspirates	56	12 (21.4%)	75	14 (18.7%)
Urine	139	10 (7.2%)	13	5 (38.5%)
Hemoculture	4	2	8	4
Wound	2	1	1	0
Others*	11	1 (9.09%)	6	2
Total	**212**	**26 (11.8%)**	**103**	**25 (24.3%)**

* Eye, umbilicus, sputum, tubus, cerebrospinal fluid.

ESBL-producing *E. coli* isolates were most commonly recovered from the respiratory tract (21.4%), followed by urine (7.2%). The percent of ESBL-positive *E. coli* strains was 50 recovered from blood and wounds, but the total number of those samples was low. Considering other samples, only one ESBL-positive *E. coli* strain was recovered from nose swab (Table 1).

ESBL-positive *K. pneumoniae* was most commonly recovered from urine (38.5%), followed by the respiratory tract (18.7%). The percent of ESBL-positive *K. pneumoniae* strains was 50 recovered from blood, but the number of those samples was low. There's no ESBL-positive *K. pneumoniae* strain recovered from wound. Considering other samples, only two ESBL-positive *K. pneumoniae* strains were recovered from nose swab and cerebrospinal fluid.

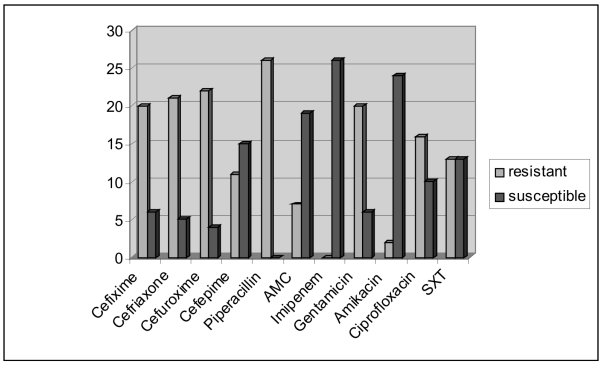

Figure 1: Antibiotic susceptibility pattern of ESBL-producing E. coli.

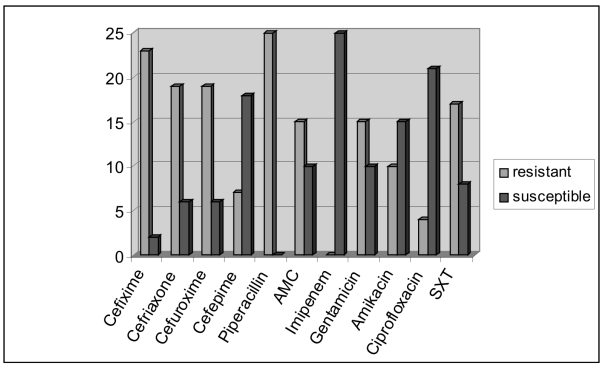

Figure 2: Antibiotic susceptibility pattern of ESBL-producing K. pneumoniae isolates.

ESBL-positive *E. coli* isolates were more susceptible to AMC (76%) and SXT (50%), than were the isolates of ESBL-positive *K. pneumoniae* (40% and 32%). Considering aminoglycosides, 92% of ESBL-positive *E. coli* and 60% of ESBL-positive *K. pneumoniae* were susceptible to amikacin vs. 23% and 40% of ESBL-positive *E. coli* and *K. pneumoniae* strains to gentamicin. Upon the determination of susceptibility to quinolones, ESBL-positive *K. pneumoniae* were more susceptible to ciprofloxacin (84%) than ESBL-positive *E. coli* (38%). Imipenem showed the best in vitro activity of all tested beta-lactams. All isolates were susceptible to imipenem (Table 2).

Table 2: Antibiotic susceptibility pattern of ESBL-producing *E. coli* and *K. pneumoniae* isolates.

Antimicrobial agent	ESBL-positive *E. coli* (N=26)		ESBL-positive *K. pneumoniae* (N=25)	
	R	S	R	S
Cefixime	20	6 (23%)	23	2 (8%)
Cefriaxone	21	5 (19%)	19	6 (24%)
Cefuroxime	22	4 (15%)	19	6 (24%)
Cefepime	11	15 (58%)	7	18 (72%)
Piperacillin	26	0	25	0
AMC	7	19 (76%)	15	10 (40%)
Imipenem	0	26 (100%)	0	25 (100%)
Gentamicin	20	6 (23%)	15	10 (40%)
Amikacin	2	24 (92%)	10	15 (60%)
Ciprofloxacin	16	10 (38%)	4	21 (84%)
SXT	13	13 (50%)	17	8 (32%)
Pipemidic acid	8	2	4	1
Nitrofurantoin	1	9	2	3
Ofloxacin	8	2	3	2
Norfloxacin	9	1	3	2

N- total number of isolates; R- resistant; S- susceptible and intermediate susceptible strains.

Considering cephalosporins, susceptibility was in the range of 15% to 58% for ESBL-positive *E. coli* and from 8% to 72% for ESBL-positive *K. pneumoniae*. Cefepime shows the best in vitro activity of all tested cephalosporins for both ESBL-positive *E. c*oli and *K. pneumoniae.*

All 15 ESBL-positive isolates from urine (10- *E. coli*, 5- *K. pneumoniae*) were tested to 15 antimicrobial agents (11 as other isolates and four additional antimicrobial agents: pipemidic acid, nitrofurantoin, ofloxacin and norfloxacin). Their susceptibility to additional agents is shown on Fig 3

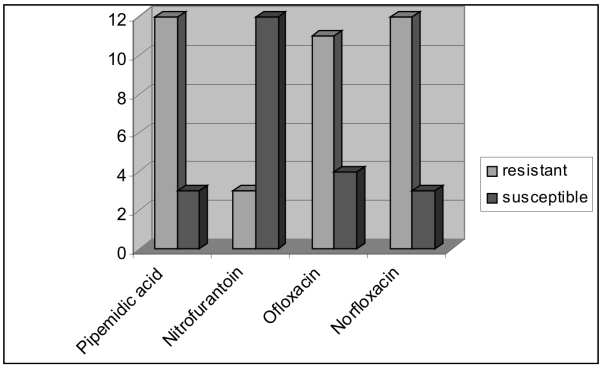

Figure 3: Antibiotic susceptibility pattern of ESBL-producing E. co*li and* K. pneumoniae *isolated from urine to four additional antimicrobial agents.*

Upon the examination of uroanticeptics, 80% of ESBL-producing organisms were susceptible to nitrofurantoin**.**

Discussion

The prevalence of ESBL-producing organisms is increasing worldwide. There's a wide range in the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* isolates between different countries. The SENTRY Antimicrobial Surveillance Program showed that ESBL-producing *K. pneumoniae* isolates were more prevalent in Latin America (45,5%), followed by the Western Pacific region (24,6%), Europe (22,6%), the United States (7,6%) and Canada (4,9%). A similar pattern was seen in ESBL-producing *E. coli* isolates although the percentage of isolates with resistance was lower (7). In Asia the prevalence of ESBL-producing *K. pneumoniae* and *E. coli* varies from 5% in Japan to 20-50% in other countries. In Europe, the prevalence of these organisms varies from country to country (3% in Sweden to 34% in Portugal) (8). Previous publications of the MYSTIC studies showed the highest rates in Russia (nearly 50%) and Poland (nearly 40%) in 2000 (9). There's no available data for the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* in our country. In this study only data from hospitalized children in Pediatric Clinic is shown. Although *E. coli* strains were more frequently isolated than *K. pneumoniae* strains, the production of ESBLs was more prevalent in *K. pneumoniae*.

In our study, the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* were with the means of 11,8% and 24,3%, respectively. These percent were lower compared to those obtained from hospitalized adult patients in different clinics of the Clinical Center in Skopje in the same period of time (15,9% of ESBL-positive *E. coli* and 32% of *K. pneumoniae*, according to unpublished data). In our study, although isolates were obtained from different departments of Pediatric Clinic (neonatology, cardiology, pulmology, nephrology, pediatric intensive care unit, immunology etc.), they were not examined separately. It'll be the aim of further research.

ESBL-producing organisms are common pathogens and isolates from various body sites. Most of the producer isolates were obtained from urinary tract infections. Lautembach at al. reported ESBL-producing *E. coli* and *K. pneumoniae* isolates were collected from urinary tract (51,5%), the wound (15,2%), the central venous catheter (12,1%), and the blood (9,1%). In hospitalized patients, ESBL-producing *E. coli* infections occurred most frequently at the surgical site (44%) and the urinary tract (17%). ESBL-positive *K. pneumoniae* isolates were most commonly obtained from the urine (51,2%), followed by the wound (22%) and the blood (19,5%) (9,10). In other study, the isolation rate of ESBL-producing *E. coli* was 42% from urine and the rate of ESBL-positive *K. pneumoniae* was 54,5%, 32% and 17% from pus, urine and sputum, respectively (11). In our study ESBL-producing *E. coli* isolates were most commonly recovered from the respiratory tract (21,4%), followed by urine (7,2%). ESBL-positive *K. pneumoniae* was most commonly recovered from urine (38,5%), followed by the respiratory tract (18,7%). The percent of ESBL-positive *E. coli* and *K. pneumoniae* strains is 50 recovered from blood, but the total number of those samples was low.

ESBL-producing organisms have become an important clinical problem due to their resistance to multiple antibiotics. Thus, antibiotic options in the treatment of these organisms are extremely limited. Beta-lactams are usually used for treatment of lower respiratory tract infections in children where Gram negative bacteria are isolated. Detection of ESBL-production is important, because it is recommended that any organism that is confirmed for ESBL production according to CLSI criteria should be reported as resistant to all extended-spectrum beta lactam antibiotics, regardless of the susceptibility test results. These drugs should not be used to treat serious infections caused by ESBL producers. Other b-lactams (b-lactam/b-lactamase inhibitor combinations or cephamycins) and non-b-lactams, such as quinolones,

aminoglycosides may be useful options to treat mild-to-moderate infections, but are not adequate as first-line therapy for serious infections (12,13).

ESBL-positive *E. coli* isolates were more susceptible to AMC (76%) and SXT (50%), than were the isolates of ESBL-positive *K. pneumoniae* (40% and 32%). Considering aminoglycosides, 92% of ESBL-positive *E. coli* and 60% of ESBL-positive *K. pneumoniae* were susceptible to amikacin vs. 23% and 40% of ESBL-positive *E. coli* and *K. pneumoniae* strains to gentamicin. Aminoglycosides showed good in vitro activity (amikacin was more active than gentamicin). But, positive clinical results may be achievable in combination of high dosage of fourth generation cephalosporin (cefepime) with an aminoglycoside. Upon the determination of susceptibility to quinolones, ESBL-positive *K. pneumoniae* were more susceptible to ciprofloxacin (84%) than ESBL-positive *E. coli* (38%). Quinolones should not be recommended for a routine usage in pediatrics, because of the toxic effect over the joint cartilage (the exception are children with cystic fibrosis, where the arthropathy is becoming rare). Other studies have reported the different percentages of susceptibility to these antibiotic agents In one study less than 50% of ESBL producing *E. coli* and *K. pneumoniae* were susceptible to amikacin, trimetoprim-sulfomethoxazole and gentamicin. Ciprofloxacin was more active against ESBL-producing *E. coli* than *K. pneumoniae* isolates. (13,14). Liao et al reported that 30 and 81,3% of ESBL-producing *E. coli* isolates, 36,6 and 72,3% of ESBL-producing *K. pneumoniae* isolates were susceptible to ciprofloxacin and amikacin (15). Upon the examination of uroanticeptics, 12 strains out of a total of 15 ESBL-positive isolates from urine (80%) were susceptible to nitrofurantoin**.** This uroantiseptic can be used only to treat lower urinary tract infections.

Carbapenems have been recommended as the drugs of choice for serious infections with ESBL-producers. The term “serious infections” generally refers to bacteriemia, hospital-acquired pneumonia, intra-abdominal infection or meningitis, excluding urinary tract infections. (12). Imipenem showed the best in vitro activity of all tested beta-lactams. In our study all ESBL producers were susceptible to imipenem. These results agree with those reported previously by other studies.

In conclusion, ESBL-producing *E. coli* and *K. pneumoniae* are present in our hospital environment. It’s necessary and useful to perform screening and confirmatory tests for phenotypic detection of those organisms in a routine work. Most of ESBL-producers are resistant to many classes of antibiotic, resulting in limited treatment options. Treatment of infections due to these organisms is difficult and complex. Therefore it’s important to control such strains in order to prevent and reduce their spread. Imipenem showed good in vitro activity against ESBL-producing strains, so it is regarded as the drug of choice in the treatment of infections caused by these bacteria (13,14).

References

1. Bradford PA. Extended-spectrum β-lactamases in 21st century: characterization, epidemiology, and detection of this important resistance threat. Clinical Microbial Reviews. 2001;14(4):933-951. doi:10.1128/CMR.14.4.933-951.2001 PMID:11585791

2. Al-Jasser AM. Extended-spectrum beta lactamases (ESBLs): a global problem. Kuwait Medical Journal. 2006; 38 (3):171-185.

3. Rahal JJ. Extended-spectrum β-lactamases: how big is the problem? Clin Microbiol Infect. 2000;6(Suppl 2):2-6. PMID:11523521

4. Gniadkowski M. Evolution and epidemiology of extended-spectrum β-lactamases (ESBLs) and ESBL-producing microorganisms. Clin Microbiol Infect. 2001;7(11):597-608. PMID:11737084

5. Nathisuwan S, Burgess SD, Lewis SJ. Extended-spectrum -lactamases: epidemiology, detection and treatment. Pharmacotherapy. 2001;21(8):920-928. doi:10.1592/phco.21.11.920.34529 PMID:11718498

6. Stürenburg E, Mack D. Extended-spectrum β-lactamases: implications for the clinical microbiology laboratory, therapy and infection control. J Infect. 2003;47(4):273-295. doi:10.1016/S0163-4453(03)00096-3 PMID:14556752

7. Winokur PL, Canton R, Casellas JM, Legakis N. Variations in the prevalence of strains expressing an extended-spectrum β–lactamase phenotype and characterization of isolates from Europe, the Americas, and the Western Pacific Region. Clin Infect Dis. 2001;32 (Suppl 2):S94-103. doi:10.1086/320182 PMID:11320450

8. Babini GS, Livermore DM. Antimicrobial resistance amongst *Klebsiella spp*. Collected from intensive care units in Southern and Western Europe in 1997-1998. J Antimicrob Chemother. 2000;45(2):183-189. doi:10.1093/jac/45.2.183 PMID:10660500

9. Canton R, Novais A, Valverde A, Machado E, Peixe L, Baquero F, Coque TM. Prevalence and spread of extended-spectrum β–lactamase-producing *Enterobacteriaceae* in Europe. Clin Microbiol Infect. 2008;14(Suppl. 1):144-153. doi:10.1111/j.1469-0691.2007.01850.x PMID:18154538

10. Lautenbach E, Patel JB, Bilker WB, Edelstein PH, Fishman NO. Extended-spectrum β–lactamase producing Escherichia coli and Klebsiella pneumoniae: risk factors for infection and impact of resistance on outcomes. Clin Infect Dis. 2001;32:1162-71. PMID: 11283805

11. Rahman MM, Haq JA, Hossain MA, Sultana R, Islam F, Islam AH. Prevalence of extended-spectrum β-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* in an urban hospital in Dhaka, Bangladesh. Int J Antimicrob Agents. 2004;24(5):508-510. doi:10.1016/j.ijantimicag.2004.05.007 PMID:15519486

12. Yu WL, Chuang YC, Walter-Rasmussen J. Extended-spectrum beta-lactamases in Taiwan: epidemiology, detection, tgreatment and infection control. J Microbiol Immunol Infect. 2006;39(4):264-77. PMID:16926972

13. Chaikittisuk N, Munsrichoom A. Extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in children at Queen Sikirit National Institute of child health. J Infect Dis Antimicrob Agents. 2007; 24(3):107-115.

14. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. Clinical Microbiolgy Reviews. 2005;18(4):657-686. doi:10.1128/CMR.18.4.657-686.2005 PMID:16223952

15. Liao CH, Sheng WH, Wang JT et al. In vitro activities of three antimicrobial agents against ESBL-producing *Escherichia co*li and *Klebsiella pneumoniae* in two regional hospitals in Taiwan. J Microbiol Immunol Infect. 2006; 39(1):59-66. doi:10.1007/3-540-32952-8_1 PMID:16440125