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Extended-spectrum β-lactamase (ESBL) in Omani Children

Study of prevalence, risk factors and clinical outcomes at Sultan Qaboos University Hospital, Sultanate of Oman

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البكتيريا التي تفرز البيتالاكتاميز ذي الطيف المديد (ESBL) في الأطفال العمانيين

دراسة الانتشار وعَوامِلُ الاِخْتِطار والنتائج السريرية في مستشفى جامعة السلطان قابوس، سلطنة عمان

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الملخص: الهدف: مقاومة البكتريا مشكلة متنامية في العالم . وهذا يضع صعوبات في اختيار المضاد الحيوي المناسب . هذه الدراسة تهدف الى تقييم البكتيريا التي تفرز البيتالاكتاميز ذي الطيف المديد (ESBL) في قسم الأطفال بمستشفى جامعة السلطان قابوس من شهر يناير إلى شهر ديسمبر 2005. **الطريقة:** خلال 12 شهرا تم تحديد ودراسة تلك البكتيريا عند الأطفال الراقدين في قسم الأطفال . تم تحليل عوامل الاختطار عند المرضى الذين أصيبوا بتلك البكتريا . **النتائج :**(13.3%) من الاشريشيا المعوية و(16.6 %) من الكليبسيلا الرئوية تفرز البيتالاكتاميز ذي الطيف المديد . غالبية هذه البكتريا (46.2 %) جاءت من البول . ومن الدم (42.6%). وأهم عوامل الاختطار التي أدت إلى الإصابة بهذه البكتيريا هو استخدام المضادات الحيوية (100%). وكلا من الترقيد الطويل في المستشفى والمرض الشديد (92.3%). وكون المريض أنثى (84.6%). كانت حساسية البكتريا للكاربابينم (100%) . و للأميكاسين (92 %) . بينما أبدى جميعها (100 %) مقاومة ضد الأوكسي- أمينو- سيفالوسبورين. لم تسجل أية مناعة ضد التوليفات التالية: الأميكاسين مع البيبراسيلين- تازوباكتم. أميكاسين مع النايتروفرونتوين. الجنتاميسين مع النايتروفرونتوين. **الخلاصة:** تمثل البكتيريا التي تفرز البيتالاكتاميز ذي الطيف المديد مشكلة كبيرة عند الأطفال المرضى العمانيين . وأن التعرض المسبق للمضادات الحيوية والترقيد الطويل هي عوامل يمكن التحكم بها لتقليل انتشار هذه البكتيريا. العلاج الأمثل لهذه البكتريا هو الكاربابينم . ويمكن الاستعاضة عنه **بالتوليفات التالية:** الأميكاسين مع البيبراسيلين- تازوباكتم. أميكاسين مع النايتروفرونتوين. الجنتاميسين مع النايتروفرونتوين.

مفتاح الكلمات: البيتالاكتاميز ذي الطيف المديد. الاشريشيا المعوية . الكليبسيلا الرئوية. عوامل الاختطار. المضادات الحيوية. عمان.

ABSTRACT ***Objectives:*** Antimicrobial resistance is a growing problem worldwide, which imposes difficulties in the selection of appropriate empirical antimicrobial therapy. This study evaluated extended-spectrum β-lactamase (ESBL) isolates in 2005 in The Department of Child Health at Sultan Qaboos University Hospital (SQUH), Oman. ***Methods:*** During the 12 month period from January 2005 to December 2005, ESBL isolates from paediatrics inpatients were identified and analysed. Risk factors for the patients who grew ESBLs were analysed. ***Results:*** 13.3% of *E. coli* and 16.6% of *Klebsiella pneumoniae* isolated were ESBL producers. Most of the ESBLs were from urine (46.2%) and blood (42.6%). The main risk factors for ESBL in these children were previous exposure to antimicrobials (100%), prolonged hospital stay, severe illness (92.3%) and female gender (84.6%). Sensitivity of 100% was observed to carbapenems whereas 92% of the isolates were susceptible to amikacin. The oximino-cephalosporins were 100% resistant. *Klebsiella pneumoniae* were 100% resistant to piperacillin-tazobactam and nitrofurantoin. *E. coli* was 100% resistant to trimethoprim-sulfamethoxazole and ciprofloxacin. No resistance was recorded for the following combinations: amikacin plus piperacillin-tazobactam, amikacin plus nitrofurantoin and gentamicin plus nitrofurantoin. ***Conclusion:*** ESBL-producing organisms are becoming a major problem in Omani children. Exposure to antimicrobials and long admissions are modifiable risk factors that should be targeted for better control. Carbapenems are the most sensitive and reliable treatment options for infections caused by ESBLs. Amikacin plus piperacillin-tazobactam or nitrofurantoin are good alternatives.

Keywords: Extended-spectrum β-lactamase; Escherichia coli; Klebsiella pneumonia; Anti-infective agents; Risk factors; Oman.

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Antimicrobial resistance is a growing problem worldwide, which imposes difficulties in the selection of appropriate empirical antimicrobial therapy. Since the first extended-spectrum β-lactamase (ESBL) producing *Klebsiella pneumoniae* were discovered in Western Europe in the mid-1980s, the ESBL producing *Enterobacteriacea* became the focus of many scientific research studies and investigations.[1-5] ESBL are enzymes belonging to either class A or class D β-lactamases. Class A ESBLs belong to three types: SHV with more than 50 varieties currently recognized on the basis of unique combinations of aminoacid replacements; TEM with more than 130 TEM enzymes currently recognized; and CTX-M with more than 40 CTX-M enzymes currently known.[6] Other uncommon class A ESBLs are BES-1, GES-1, GES-2, IBC-1, IBC-2, PER-1, SFO-1, TLA-1, VEB-1 and VEB-2.[6] There are also at least twelve ESBLs belonging to the OXA type (class D).[6] ESBLs are plasmid-mediated, and their potential for transfer makes it increasingly difficult to control and treat these organisms effectively.[7] As of 25 January 2005, there were 138 TEM- (TEM-1 to TEM-139) and 62 SHV-types (SHV-1 to SHV-63) of β-lactamases, mostly found in *K. pneumoniae* and *E. coli* strains.[8] These mutant enzymes were termed 'Extended-Spectrum β-Lactamase' by Philippon et al[9] in 1989. ESBLs hydrolyse extended spectrum cephalosporins with an oxyimino side chain.[10] These cephalosporins include cefotaxime, ceftriaxone and ceftazidime, as well as the oxyimino-monobactam aztreonam. In addition, ESBL-producing organisms are frequently resistant to many other classes of antibiotics, including fluoroquinolones, the monobactam aztreonam, while resistance to trimethoprim–sulfamethoxazole and aminoglycosides is frequently co-transferred on the same plasmid.[9, 11-14] ESBLs are sensitive to cephamycins (cefoxitin, cefotetan) and carbapenems. ESBL-producing organisms are poorly responsive to treatment with wide spectrum cephalosporins such as ceftazidime and cefepime.[7, 15] ESBL-producing organisms are difficult to differentiate from AmpC β-lactamase-producing *Enterobacteriacea*; however, most ESBL producers are generally susceptible to cephamycins (e.g. cefoxitin) in vitro. ESBLs are plasmid-mediated while AmpC β-lactamase enzymes are located on the chromosomes of *Enterobacter sp*, *Citrobacter freundii, Morganella morganii, Serratia marcescens,* and *Pseudomonas aeruginosa.* The appearance of similar plasmid-mediated β-lactamases in *K. pneumoniae* and *E. coli* raises concerns over the spread of resistance,[7] which will further increase the difficulties of phenotypically identifying ß-lactamases.[16-21]

There are many precipitating factors for selection of ESBL producing organisms. These include the increasing use of oxyimino-β-lactams such as ceftazidime, cefotaxime and ceftriaxone. Other risk factors for the acquisition of ESBLs include presence of intravascular catheters; emergency intra-abdominal surgery; a gastrostomy or jejunostomy tube; gastrointestinal colonisation; length of hospital or intensive care unit stay; prior antibiotics (including third-generation cephalosporins); prior nursing home stay; severity of illness; presence of a urinary catheter and ventilator assistance.[22]

The problem of ESBL production is still relatively unappreciated by most clinicians.[23, 24] This may be due in part to difficulty in laboratory identification of ESBLs and misreporting them as sensitive organisms.[15, 25-27] Many ESBL-producing isolates are not always phenotypically resistant to all oximino-cephalosporins; however, patients suffering from infections caused by ESBL-producing organisms are at risk of treatment failure if treated with one of the oximino-cephalosporins.[9, 28, 29] Therefore, it is imperative for the clinical microbiology laboratory to identify isolates that possess increased minimum inhibitory concentrations (MICs) (≥ 2 μg/mL) to oximino-cephalosporins, even though they may be equal to or below the susceptibility breakpoint (MIC ≤ 8 μg/mL).[9]

The rate of ESBL varies from country to country. The prevalence of ESBLs in the UK in 2002 was 7.4%.[30] In Europe, the prevalence of ESBL producing *E.coli* is 10.8% while *K. pneumoniae* is 13.6%.[31] In the USA, the prevalence of ESBL producing *E.coli* is 1.4% while *K. pneumoniae* is 4.4%[31]. The prevalence of ESBL at Sultan Qaboos University Hospital, (SQUH), Oman is not yet known. We have analysed the sensitivity and distribution of some ESBL isolates in SQUH previously[32] without studying the prevalence rate of ESBL in SQUH as a whole, or in individual departments. In this article, we are reporting the prevalence of ESBL isolates in paediatric patients admitted to SQUH with an analysis of the risk factors and clinical outcomes of ESBLs infections.

Table 1: ***Percentage of ESBLs among E.coli and K. pneumoniae in Sultan Qaboos University Hospital, Oman, paediatrics wards in 2005***

Isolates	Total	ESBLs	ESBL (%)
E.coli	45	6	13.3
K. pneumoniae	42	7	16.6
Total	87	13	14.9

METHODS

SQUH is a 500-bed tertiary and teaching hospital covering all major medical specialties. It is located on the campus of Sultan Qaboos University in Muscat, Oman. The Department of Child Health occupies three different wards. Each ward accommodates 24 beds of which 4 beds are for isolation.

All specimens received from Department of Child Health from January-December 2005 were properly processed to identify ESBLs. Initially, the isolates were screened by a commercial system (Phoenix Identification and Susceptibility System from Becton Dickinson) for ESBL production. The positive results were further confirmed using the Clinical and Laboratory Standards Institute (CLSI) approved double-disk diffusion method[14], which is based on a synergistic increase of inhibition zone of ceftazidime and cefotaxime when they are combined with clavulanate. The test is considered positive when the increase of the inhibition zone is (>/= 5 mm).[9, 33, 34]

Susceptibility results were recorded for the following antimicrobials using the Phoenix Identification and Susceptibility System: gentamicin, amikacin, imipenem, meropenem, cefotaxime, ceftazidime, cefepime, ciprofloxacin, piperacillin-tazobactam, trimethoprim/sulfamethoxazole and nitrofurantoin.

There was no resistance recorded for the following combinations: amikacin plus piperacillin-tazobactam, amikacin plus nitrofurantoin and gentamicin plus nitrofurantoin [Table 5].

RESULTS

A total of 87 isolates of *E. coli* and *Klebsiella pneumoniae* were isolated from patients admitted to paediatric wards in SQUH in 2005. Out of these 13 (14.9 %) were ESBL producers, out of which 6 (46.2%) were *E. coli* and 7 (53.8%) were *Klebsiella pneumoniae* [Table 1]. The percentage of *E. coli* producing ESBL from the total number of *E. coli* isolated in 2005 was 13.3% [Table 1]. While the percentage of *Klebsiella pneumoniae* producing ESBL from the total number of *Klebsiella pneumoniae* isolated in 2005 was 16.6% [Table 1].

Most of the ESBLs isolates were from urine (46.2%) and blood (42.6%) [Table 2]. A total of 85.7% of ESBL producing *Klebsiella pneumoniae* were isolated from urine samples, while 83.3% of ESBL producing *E. coli* were from blood [Table 1]. No ESBL were isolated from wound and pus swabs.

The main risk factors for ESBL in these children were the previous exposure to antimicrobials (100%) [Table 3], hospital stays of more than 5 days (92.3%) and female sex (84.6%). Malignancies, admission to the Intensive Care Unit and the use of a urinary catheter were each (38.5%) associated with ESBL. Only one patient (1/13) was ventilated. Abdominal surgery and obstructive disease of the urinary tract were not found to be risk factors in our patients.

The carbapenems (imipenem and meropenem) were the most active antibiotics against the ESBLs tested, with no resistance recorded [Table 4], followed by amikacin with 8% resistance. All the ESBLs were resistant to oximino-cephalosporins.

All ESBL producing *Klebsiella pneumoniae* were sensitive to gentamycin and amikacin [Table 4], whereas all *E. coli* were resistant to gentamycin and but only 18% were resistant to amikacin.

All *Klebsiella pneumoniae* were resistant to piperacillin-tazobactam and nitrofurantoin, whereas no resistance was seen in *E. coli* to nitrofurantoin and only 16% were resistant to piperacillin-tazobactam. All *E. coli* were resistant to ciprofloxacin and trimethoprim/sulfamethoxazole, while 14% of *Klebsiella pneumoniae*

Table 2: ***Source of ESBL isolates from paediatric wards***

Isolates	Blood	Respiratory	Swabs	Urine
Klebsiella pnemoniae	1	0	0	6
E. coli	5	1	0	0
Total	6	1	0	6

Table 3: ***ESBL risk factors in a group of Sultan Qaboos University Hospital paediatric patients***

Risk factors	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10	Case 11	Case 12	Case 13	Total
Sex	M	F	F	M	F	F	F	F	F	F	F	F	F	F=11 M=2
Previous use of antibiotics	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	13/13
Hospital stay > 5 days	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	12/13
Malignancies	N	Y	N	N	N	Y	Y	N	Y	N	N	Y	N	5/13
Use of urinary catheter	N	N	Y	N	N	N	N	Y	N	Y	Y	N	Y	5/13
Admission to Intensive Care Unit	Y	N	N	N	N	Y	Y	N	Y	N	N	Y	N	5/13
Ventilatory assistance	Y	N	N	N	N	N	N	N	N	N	N	N	N	1/13
Abdominal surgery	N	N	N	N	N	N	N	N	N	N	N	N	N	0
Obstructive disease of urinary tract	N	N	N	N	N	N	N	N	N	N	N	N	N	0

were resistant to ciprofloxacin and 28% were resistant to trimethoprim/sulfamethoxazole [Table 4].

No resistance was recorded for the following combinations: amikacin plus piperacillin-tazobactam, amikacin plus nitrofurantoin and gentamicin plus nitrofurantoin [Table 5]. *Klebsiella pneumoniae* isolates were sensitive to all combinations containing gentamicin and amikacin [Table 5]. *E. coli* isolates were sensitive to all combination containing nitrofurantoin [Table 5].

All patients (100%) were isolated in a single room and nursed using gloves. They were all treated with a carbapenem (imipenem or meropenem). All patients (100%) cleared the infection.

DISCUSSION

The percentage of ESBL producing *E. coli* and *Klebsiella pneumoniae* in children admitted at SQUH was low compared to other SQUH wards. The prevalence of ESBLs in medical wards was 28.3% (unpublished data); however, the prevalence rate of ESBLs among *E. coli* and *Klebsiella pneumoniae* isolated from paediatrics patients was significantly high (13.3% and 16.6% respectively) compared to the prevalence of ESBLs in the USA in 2004 (1.4% for *E. coli* and 4.4% *Klebsiella pneumoniae*)[31] and Europe (10.8% for *E. coli* and 13.6% for *Klebsiella pneumoniae*).[31] The rate of ESBL among *E. coli* (13.3%) was lower than that for *Klebsiella pneumoniae* (16.6%). This was the same as the prevalence in USA (1.4% for *E. coli* versus 4.4% for *Klebsiella pneumoniae*) and Europe (10.8% for *E. coli* versus 13.6% for *Klebsiella pneumoniae*).[31]

Urine (70.8%) was the main source of ESBLs from all patients, followed by blood (15%). The high rate of ESBLs in urine samples is not striking if we consider the high prevalence of ESBLs in the gut as shown in Hong Kong where the faecal carriage rates of ESBL is 19% in general outpatients, 19.3% in hospitalized patients, 22.5% in healthy inmates, and 33.3% in convalescent patients.[35] In one Middle East country, 1.25% of all gram-negative organisms causing community acquired urinary tract infections during 1999 were reported as ESBL producers.[22, 36]

Previous exposure to antimicrobials, female sex,

Table 4: ***ESBL isolates from Paediatric wards: frequency and (%)***

Isolates	CN	AK	IMI	MEM	CTX	CAZ	CEFI	CIP	TAZ	SXT	F
Klebsiella pnemoniae	0	0	0	0	7	7	7	1	7	2	7
E. coli	6	1	0	0	6	6	6	6	1	6	0
Total	**6 (46)**	**1 (8)**	**0**	**0**	**13 (100)**	**13 (100)**	**13 (100)**	**7 (54)**	**8 (62)**	**8 (62)**	**7 (54)**

CN = gentamicin, AK = amikacin, IMI = imipenem, MEM = meropenem CTX = cefotaxime, CAZ = ceftazidime, CEFI = cefepime, CIP = ciprofloxacin, TAZ = tazocin, SXT = cotrimoxazole, F = nitrofurantoin.

prolonged hospital stays of more than 5 days, malignancies, admission to the Intensive Care Unit and the use of a urinary catheter were all found to be risk factors for ESBL acquisition. Exposure to antimicrobials was present in 100% of cases, which supports the need for antimicrobial control. 84.6% of cases were female, this might be explained by the increased frequency of urinary tract infections (UTI) in females.

All the ESBL isolates in SQUH were susceptible to carbapenems (100%). The susceptibility rate is similar to ESBLs in the USA where they were 100% susceptible to meropenem and imipenem.[31] Meropenem and imipenem activity against ESBL producing *E. coli* and *Klebsiella spp.* collected in Europe during 1997–2004 was between 96.9–100.0%, which was lower than the susceptibility of ESBLs in SQUH and the USA.[31] Susceptibility of ESBLs from SQUH to amikacin was very high (95.9% for *E. coli* and 90% for *Klebsiella pneumoniae*). Kizirgila et al have shown similar susceptibility patterns to amikacin for ESBLs in Turkey (94.5 for *E. coli* and 83.3% for *Klebsiella pneumoniae*), [37] which makes amikacin a good antibiotic in treatment of ESBLs especially in combination therapy. On the contrary, gentamicin had very low activity against ESBLs at SQUH. Gentamicin had only (28.8%) activity against *E. coli* compared to Europe and USA where the *E. coli* susceptibility to gentamicin was 66.7% and 80% respectively in 2004.[31] Gentamicin had only 25% activity against *Klebsiella pneumoniae* which is similar to the USA (26.3%), which are lower rates than those (47.5%) reported in Europe in 2004.[31]

ESBLs at SQUH had low susceptibility against piperacillin/tazobactam (50.7% *E. coli* and 32.5% *Klebsiella pneumoniae*). This level was lower than that reported in Europe (72.5% *E. coli* and 38.6% *Klebsiella pneumoniae*)[31] and the USA (80.0% for *E. coli* and 42.1% for *Klebsiella pneumoniae*)[31], which does not make piperacillin/tazobactam a good empirical choice if suspicion of ESBL is high.

Ciprofloxacin had very low activity against ESBLs in SQUH. It was only 16.4% active against *E. coli* which is similar to Europe (20.2%) and the USA (20%) in 2004, [31] whereas higher activity against *Klebsiella pneumoniae* (32.5%) was recorded. This higher activity compared to *E. coli* has also been demonstrated in Europe (57.5%) and the USA (36.8%) in 2004.[31] The opposite situation has been detected in Turkey where ciprofloxacin was more active against *E. coli* (33.3%) compared to *Klebsiella pneumoniae* (25.9%).[37]

The best non-carbapenem containing combinations were amikacin plus piperacillin-tazobactam, amikacin plus nitrofurantoin and gentamycin plus nitrofurantoin. So if ESBL is expected in a severely ill patient the best empirical combination therapy would be amikacin plus piperacillin-tazobactam. If *Klebsiella pneumoniae* were cultured and a suspension of ESBL was present, the empirical combination therapy should include either gentamycin or amikacin. If *E. coli* was isolated from a urine culture of a stable patient nitrofurantoin would be the drug of choice.

All patients underwent a good infection control procedure of isolation and barrier nursing according to accepted standards. All patients made a full clinical recovery with microbiologic eradication of ESBLs on carbapenem.

Overall prevalence of ESBL-producing isolates in Omani children was high compared to other countries. Prevention and good infection control practices should be our priority because these organisms have very limited treatment options. Modification of risk factors and control of antimicrobials should be considered. Carbapenem should be the drug of choice in treatment of ESBLs, which theoretically may lead to increase in carbapenem-resistant *Acinetobacter sp* and carbapenem-resistant *P. aeruginosa*. However, Robert G et al have not seen any increase in carbapenem

Table 5: ***ESBL Resistance to combination therapy of Sultan Qaboos University Hospital paediatric patients***

Isolates	CN + CEP	CN + TAZ	CN + CIP	CN + F	AK + CEP	AK + TAZ	AK + CIP	AK+ F	CIP + TAZ	CIP + CEP	CIP + F	F + TAZ	F + CEP
Klebsiella pneumoniae	0	0	0	0	0	0	0	0	1	1	1	7	7
E. Coli	6	1	6	0	1	0	1	0	1	6	0	0	0
Total	6 (46)	1 (8)	6 (46)	0	1 (8)	0	1 (8)	0	2 (16)	7 (54)	1 (8)	7 (54)	7 (54)

CN + CEP = gentamicin+ cefepime, CN + TAZ =gentamicin+ tazocin, CN + CIP = gentamicin+ ciprofloxacin, CN + F = gentamicin + nitrofurantoin AK + CEP = amikacin + cefepime, AK + TAZ = amikacin + tazocin, AK + CIP = amikacin + ciprofloxacin, AK + F = amikacin + nitrofurantoin, CIP + TAZ = ciprofloxacin + tazocin, CIP + CEP = ciprofloxacin + cefepime, CIP + F = ciprofloxacin + nitrofurantoin, F + TAZ = nitrofurantoin + tazocin, F + CEP = nitrofurantoin + cefepime

resistance despite continued use of meropenem and imipenem.[30] Other options would be amikacin plus piperacillin-tazobactam or nitrofurantoin. Wong-Beringer suggested the use of piperacillin–tazobactam in the case of a non-outbreak situation, to preserve the therapeutic value of carbapenem.[38]

CONCLUSION

ESBL-producing organisms are becoming a major problem in Omani children. Exposure to antimicrobials and long admissions are modifiable risk factors that should be targeted for better control. Carbapenems are the most sensitive and reliable treatment options for infections caused by ESBLs. Amikacin plus piperacillin-tazobactam or nitrofurantoin are good alternatives.

REFERENCES

1. Winokur PL, Canton R, Casellas JM, Legakis N. Variations in the prevalence of strains expressing an extended spectrum b-lactamase phenotype and characterisation of isolates from Europe, the Americas and the Western Pacific region. Clin Microbiol Dis 2001; 32:S94–S103.
2. Bradford PA. Extended-spectrum b-lactamases in the 21st century: characterisation, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev 2001; 14:933–951.
3. Quinn JP, Miyashiro D, Sahm D, Flamm R, Bush K. Novel plasmid-mediated ß-Lactamase (TEM-10) conferring selective resistance to ceftazidime and aztreonam in clinical isolates of Klebsiella pneumoniae. Antimicrob Agents Chemother 1989; 33:1451-1456.
4. Knothe A, Shah P, Kremery V, Antal M, Mitsuhashi S. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime and in clinical isolates of Klebsiella pneumoniae and Serratia marcescens. Infection 1983; 11:315-317.
5. Jacoby GA, Medeiros AA, O'Brien TF, Pinto ME, Jiang J. Broad-spectrum transmissible β-Lactamases. N Engl J Med 1989; 319:723-724.
6. Jacoby GA, Munoz-Price LS. The New β-Lactamases. N Engl J Med 2005; 352:380-391.
7. Kaye KS, Engemann JJ, Fraimow HS, Abrutyn E. Pathogens resistant to antimicrobial agents: epidemiology, molecular mechanisms, and clinical management. Infect Dis Clin North Am 2004; 18:467– 511.
8. Giamarellou H. Multidrug resistance in Gram-negative bacteria that produce extended-spectrum b-lactamases (ESBLs). Clin Microbiol & Infection 2005; 11:1-16
9. Philippon A, Labia R, Jacoby G. Extended-spectrum b-lactamases. Antimicrob Agents Chemother 1989; 33:1131–1136.
10. Patterson JE. Extended-spectrum beta-lactamases. Semin Respir Infect 2000; 15:299-307.
11. Itokazu GS, Quinn JP, Bell-Dixon C, Kahan FM, Weinstein RA. Antimicrobial resistance rates among aerobic gram-negative bacilli recovered from patients in intensive care units: evaluation of a national postmarketing surveillance program. Clin Infect Dis 1996; 23:779-784.
12. Jacoby GA, Sutton L. Properties of plasmids responsible for production of extended-spectrum ß-Lactamases. Antimicrob Agents Chemother 1991; 35:164.
13. Jacoby GA. Genetics of extended-spectrum ß-Lactamases. Eur J Clin Infect Dis 1994; 13:2-11.
14. Rice LB, Willey SH, Papanicolaou GA, Medeiros AA, Eliopoulos GM, Moellering RC Jr, et al. Outbreak of ceftazidime resistance caused by extended-spectrum b-lactamases at a Massachusetts chronic-care facility. Antimicrob Agents Chemother 1990; 11:2193–2199.
15. Paterson DL, Ko WC, Von Gottberg A, Casellas JM, Mulazimoglu L, Klugman KP, et al. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum b-lactamases: implications for the clini-

cal microbiology laboratory. J Clin Microbiol 2001; 39:2206–2212.

16. Coudron PE, Moland ES, Thomson KS. Occurrence and detection of AmpC ß-lactamases among Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis isolates at a veteran's medical center. J Clin Microbiol 2000; 38:1791-1796.

17. Bauernfeind A, Wagner S, Jungwirth R, Schneider I, Meyer D. A novel class C ß-lactamase (FOX-2) in Escherichia coli conferring resistance to cephamycins. Antimicrob Agents Chemother 1997; 41:2041-2046.

18. Bou G, Oliver A, Ojeda M, Monzon C, Martinez-Beltran J. Molecular characterization of FOX-4, a new AmpC-type plasmid-mediated ß-lactamase from an Escherichia coli strain isolated in Spain. Antimicrob Agents Chemother 2000; 44:2549-2553.

19. Gazouli M, Tzouvelekis LS, Vatopoulos AC, Tzelepi E. Transferrable class C ß-lactamases in Escherichia coli strains isolated in Greek hospitals and characterization of two enzyme variants (LAT-3 and LAT-4) closely related to Citrobacter freundii AmpC ß-lactamase. J Antimicrob Chemother 1998; 42:419-425.

20. Fortineau N, Poirel L, Nordmann P. Plasmid-mediated and inducible cephalosporinase DHA-2 from Klebsiella pneumoniae. J Antimicrob Chemother 2001; 47:207-210.

21. Queenan AM, Jenkins S, Bush K. Cloning and biochemical characterization of FOX-5, an AmpC-type plasmid-encoded ß-lactamase from a New York City Klebsiella pneumoniae clinical isolate. Antimicrob Agents Chemother 2001; 45:3189-3194.

22. Colodner R, Extended-spectrum b-lactamases: A challenge for clinical microbiologists and infection control specialists. Am J Infection Control 2005; 2:104-107.

23. Paterson DL, Yu VL. Extended-spectrum beta-lactamases: a call for improved detection and control. Clin Infect Dis 1999; 29:1419-1422.

24. Goossens H. The problem of ESBL producers - A worldwide survey into physician awareness and perception. Program and abstracts of the 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, California, USA, 27-30 September 2002.

25. Steward CD, Rasheed JK, Hubert SK, Biddle JW, Raney PM, Anderson GJ, et al. Characterization of clinical isolates of Klebsiella pneumoniae from 19 laboratories using the National Committee for Clinical Laboratory Standards extended-spectrum beta-lactamase detection methods. J Clin Microbiol 2001; 39:2864-2872.

26. Tenover FC. ESBL testing, interpretation, and reporting. Program and abstracts of the 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, California, USA, 27-30 September 2002.

27. Document M100-S14. Clinical and Laboratory Standards Institute, formerly National Committee for Clinical Laboratory Standards. Wayne, PA: NCCLS, 2004.

28. Paterson DL, Singh N, Gayowski T, Marino IR. Fatal infection due to extended-spectrum beta-lactamase-producing Escherichia coli: implications for antibiotic choice for spontaneous bacterial peritonitis. Clin Infect Dis 1999; 28:683–684.

29. Karas JA, Pillay DG, Muckart D, Sturm AW. Treatment failure due to extended spectrum b-lactamase. J Antimicrob Chemother 1996; 37:203–204.

30. Robert G. Masterton, Philip J. Turner. Trends in antimicrobial susceptibility in UK centres: the MYSTIC Programme (1997–2002). Int J Antimicrob Agents 2006; 27:69–72.

31. Goossens H, Grabein B. Prevalence and antimicrobial susceptibility data for extended-spectrum β-lactamase– and AmpC-producing Enterobacteriaceae from the MYSTIC Program in Europe and the United States (1997–2004). Diagn Microbiol Infect Dis 2005; 53:257–264.

32. Rafay AM, Almuharrmi Z, Toki R. Prevalence of extended–spectrum beta-Lactamases-producing isolates over a one-year period at a University Hospital in Oman. Saudi Med J 2007; 28: 22-27.

33. Carter MW, Oakton KJ, Warner M, Livermore DM. Detection of extended-spectrum b-lactamases in Klebsiella with the Oxoid combination disk method. J Clin Microbiol 2000; 38:4228–4232.

34. M'Zali FH, Chanawong A, Kerr KG, Birkenhead D, Hawkey PM. Detection of extended-spectrum b-lactamases in members of the family Enterobacteriaceae: comparison of the MAST DD test, the double disc and the Etest ESBL. J Antimicrob Chemother 2000; 45:881–885.

35. Boost M, Tsang KL, Kam KM. ESBL-Producing Strains of E. coli in Hong Kong. Int J Antimicrob Agents 2005; 26S:S65- S112.

36. Colodner R, Keness Y, Chazan B, Raz R. Antimicrobial susceptibility of Community acquired uropathogens in Northern Israel. Int J Antimicrob Agents 2001; 18:189-92.

37. Kizirgila A, Demirdagb K, Ozdenb M, Buluta Y, Yakupogullaria Y, Toramana ZA. In vitro activity of three different antimicrobial agents against ESBL producing Escherichia coli and Klebsiella pneumoniae blood isolates. Microbiol Res 2005; 160:135-140.

38. Wong-Beringer A, Hindler J, Loeloff M, Queenan AM, Lee N, Pegues DA, et al. Molecular correlation for the treatment outcomes in bloodstream infections caused by Escherichia coli and Klebsiella pneumoniae with reduced susceptibility to ceftazidime. Clin Infect Dis 2002; 34:135-46.