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Original

Prevalence and susceptibility patterns of extended-spectrum betalactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in a general university hospital in Beirut, Lebanon

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SUMMARY

Extended-spectrum betalactamases (ESBLs) are recognized worldwide as a problem in hospitalized patients. Their prevalence among clinical isolates of Enterobacteriaceae *varies between countries and institutions. We studied the evolution of ESBL production by clinical isolates of* Escherichia coli *and* Klebsiella pneumoniae *and analyzed the patterns of susceptibility of these isolates to different antimicrobial agents in a general university hospital in Beirut. Of the 4299 isolates of* E. coli *and 1248 isolates of* K. pneumoniae *tested over the five years, 2.0% of the* E. coli *and 20.0% of* K. pneumoniae *were ESBL producing. A clear decrease in the susceptibility to all antibiotics was observed between 1999 and 2001, and no resistance to imipenem was detected. The isolates were distributed between the Intensive Care Unit (ICU), medical wards, outpatients, and other origins. The highest numbers were found in the ICU (*E. coli *28.1% and* K. pneumoniae *34.8%). Three phenotypes of resistance to cefotaxime and ceftazidime were observed on the basis of microbiological results. The present study was the first to assess the occurrence and susceptibility patterns of extended-spectrum betalactamase-producing* Enterobacteriaceae *in Lebanon.*

Key words: Extended spectrum betalactamase - *Escherichia coli - Klebsiella pneumoniae* - Resistance

Prevalencia y patrones de sensibilidad de Escherichia coli *y* Klebsiella pneumoniae *productoras de betalactamasas de espectro ampliado en Beirut, Líbano*

RESUMEN

Las betalactamasas de espectro ampliado (BLEA) representan un problema de ámbito mundial en los pacientes hospitalizados. Su prevalencia entre las cepas clínicas de Enterobacteriaceae *varía según los países y las instituciones. Estudiamos la evolución de la producción de BLEA por cepas clínicas de* Escherichia coli *y* Klebsiella pneumoniae *y analizamos sus patrones de sensibilidad a distintos agentes antimicrobianos en un hospital general universitario en Beirut. De las 4299 cepas de* E. coli *y las 1248 de* K. pneumoniae *analizadas durante cinco años, un 2% de las cepas de* E. coli *y un 20% de las de* K. pneumoniae *fueron productoras de BLEA. Entre 1999 y 2001 se observó una clara disminución de la sensibilidad frente a todos los antibióticos y no se detectó ninguna resistencia al imipenem. Las cepas fueron aisladas en la Unidad de Cuidados Intensivos (UCI), las salas hospitalarias, las consultas externas y otros lugares. La mayor cantidad de cepas se aisló en la UCI (un 28,1% de* E. coli *y un 34,8% de* K. pneumoniae*). En el estudio microbiológico se identificaron tres fenotipos de resistencia a cefotaxima y ceftazidima. El presente estudio es el primero en evaluar la existencia y los patrones de sensibilidad de enterobacterias productoras de BLEA en Líbano.*

Key words: Betalactamasas de amplio espectro – *Escherichia coli – Klebsiella pneumoniae* – Resistencia

INTRODUCTION

Extended-spectrum betalactamase (ESBL) is recognized worldwide as a problem in hospitalized patients. This phenomenon of resistance was first detected in Germany and then in France, most likely due to the initial use of extended-spectrum betalactam antibiotics in those specific areas. This resistance to third generation cephalosporins was then detected in the United States and Asia. The prevalence of ESBL among clinical isolates varies between countries and institutions. In the United States, occurrence of ESBL production in *Enterobacteriaceae* ranges from 0-25%, depending on the institution, with the national average being around 3% (Centers for Disease Control National Nosocomial Infections Surveillance). In Europe, the prevalence of ESBL production among isolates of *Enterobacteriaceae* varies greatly from country to country. In the Netherlands, a survey of 11 hospital laboratories showed that less than 1% of *Escherichia coli* and *Klebsiella pneumoniae* strains produced ESBL (1). In France, 40% of *K. pneumoniae* isolates were found to be ceftazidime resistant (2, 3). ESBL can be produced by various bacterial strains mainly by *E. coli, K. pneumoniae, Citrobacter, Enterobacter, Morganella, Proteus, Providencia, Salmonella* and *Shigella* (4, 5).

The microbiology laboratory plays an important role in detecting and promptly reporting the isolation of ESBL-producing strains, as well as in providing the clinician with reliable therapeutic options for successful treatment. These options should focus on antimicrobial classes other than betalactams.

The high volume and indiscriminate use of extended-spectrum cephalosporins is a common practice in all hospitals with high prevalence of ESBL production (6, 7). Specific risk factors include length of hospital stay, severity of illness, time in the ICU, intubation and mechanical ventilation, urinary or arterial catheterization, and previous exposure to antibiotics (6, 8).

In Lebanon, although a national study for ESBL prevalence is still lacking, some reports have highlighted the increasing percentage of these enzymes among *E. coli* and *K. pneumoniae* (9). The wide and inappropriate use of extended spectrum cephalosporins prompted us to carry out this survey over a period of five years. Our objective was to study the evolution of ESBL production by the clinical isolates of *E. coli* and *K. pneumoniae* and to analyze the patterns of susceptibility of these isolates to different antimicrobial agents.

MATERIALS AND METHODS

Selection of clinical isolates and patients

From January 1997 to December 2001, a total of 6532 *E. coli* isolates and 2197 *K. pneumoniae* isolates were collected and identified in the Clinical Microbiology Laboratory of the Saint George University Hospital, a 300-bed hospital in Beirut. A total of 4299 isolates of *E. coli* and 1248 isolates of *K. pneumoniae*, including those from different patients or those with different susceptibilities from the same patient, were selected for the study. Repeat isolates were excluded; these were defined as an isolate that was the same species with the same susceptibility profile and from the same site as an isolate recovered from another specimen from the same patient earlier in the 5-year study period. These strains were identified by using standard techniques (10) and/or the API 20E system (BioMérieux, Marcy l'Etoile, France).

Susceptibility test and extended-spectrum betalactamase production detection

Antimicrobial susceptibility testing of all antibiotics was carried out using the Kirby-Bauer disk diffusion method, and susceptibility was determined according to the National Committee for Clinical Laboratory Standards (NCCLS) (11). To detect ESBL production, we used the double-disk approximation test described by Jarlier *et al.* (12). In this test, the organism was swabbed onto a Mueller-Hinton agar plate. A susceptibility disk containing amoxicillin-clavulanic acid was placed in the center of the plate, and disks of ceftazidime, cefotaxime, and ceftriaxone were placed 30-35 mm (center to center) from the amoxicillin-clavulanic acid disk. Enhancement of the area of inhibition of the oxyamino-betalactam caused by the synergy of the clavulanic acid in the amoxicillin-clavulanic acid disk was considered a positive result; therefore, the strain was reported as nonsusceptible to all extended-spectrum cephalosporins and aztreonam, regardless of the susceptibility testing result (13). The antimicrobial agents and their sources were as follows: ampicillin and cefoxitin (Sigma Chemical Company); aztreonam (Bristol-Myers Squibb); ceftazidime (Glaxo Group Research); cefotaxime and cefuroxime (Hoechst-Roussel Pharmaceuticals); ceftriaxone (Hoffmann-La Roche); amoxicillin-clavulanic acid (Glaxo SmithKline); piperacillin and piperacillin-tazobactam (Lederle); cephalothin; cefepime (Bristol, Myers Squibb); imipenem (Merck Sharp and Dohme); gentamicin (Beecham); tobramycin (Eli Lilly and Company);

Table 1. Prevalence of extended-spectrum betalactamase (ESBL)-producing *E. coli* and *K. pneumoniae* isolates between 1997 and 2001.

	E. coli			*K. pneumoniae*		
	No. isolated strains	No. ESBL-producing strains (%)	No. patients	No. isolated strains	No. ESBL-producing strains (%)	No. patients
1997	560	7 (1.3)	6	120	9 (7.50)	9
1998	771	7 (0.9)	7	267	38 (14.2)	36
1999	951	11 (1.2)	8	203	40 (19.7)	38
2000	1214	28 (2.3)	26	458	110 (24.0)	108
2001	803	36 (4.0)	30	200	47 (24.0)	121
Total	4299	89 (2.0)	77	1248	244 (20.0)	312

Table 2. Number of extended-spectrum betalactamase-positive strains of *E. coli* and *K. pneumoniae* isolated yearly from the different clinical specimens.

	Type of specimen										
	Urine		Blood		Respiratory		Wound		Others		
Year	*E. coli*	*K. pneumoniae*	*E. coli*	*K. pneumoniae*	*E. coli*	*K. pneumoniae*	*E. coli*	*K. pneumoniae*	*E. coli*	*K. pneumoniae*	Total
1997	3	5	2	3	1	0	1	0	0	1	16
1998	5	33	0	3	0	2	1	0	1	0	45
1999	10	16	0	8	0	8	1	3	0	5	51
2000	21	70	3	4	1	4	3	23	0	9	138
2001	22	30	7	8	2	7	3	1	2	1	83
Total	61	154	12	26	4	21	9	27	3	16	333

amikacin (Geneva Pharmaceuticals, Novartis); norfloxacin (Merck); ofloxacin (Johnson & Johnson); pefloxacin (Rhône-Poulenc Rorer); and ciprofloxacin (Bayer).

RESULTS

Occurrence of extended-spectrum betalactamase-producing organisms

Of the 4299 isolates of *E. coli* and 1248 isolates of *K. pneumoniae* tested over the 5 years, enhanced areas of inhibition were observed with 89 isolates of *E. coli* and 244 isolates of *K. pneumoniae*; therefore, 2.0% of the *E. coli* and 20.0% of the *K. pneumoniae* were ESBL-producing organisms. Table 1 shows the number of isolates of each species that produced ESBLs per year. The 333 ESBL-producing isolates were recovered from the following specimens: 215 in urine, 38 in blood, 25 in respiratory fluids, 36 in wounds, and 19 others (Table 2). A repeat isolate was defined as an isolate of the same species and susceptibility profile taken from the same site as an isolate from another specimen recovered from the same patient on a previous occasion in the 5-year study period, and both isolates were ESBL producing. The total number of patients infected with ESBL strains was 389.

The percentage of ESBL-producing *K. pneumoniae* showed a continuous increasing trend over the 5-year period. It was 7.5% in 1997, 14.2% in 1998, 19.7% in 1999 and reached a maximum of 24% in the years 2000 and 2001. Although the numbers with *E. coli* showed lower incidence of ESBL-producing strains, a consistent increase was observed with a maximum of 4% in 2001.

When comparing the last two years, a significant decrease was observed in the year 2001 in the occurrence of ESBL-producing *K. pneumoniae* from urine and *E. coli* from wound specimens.

Susceptibility patterns and inhospital distribution

Figures 1 and 2 show the percentages of susceptibility of ESBL-producing strains. A clear decrease in the suscep-

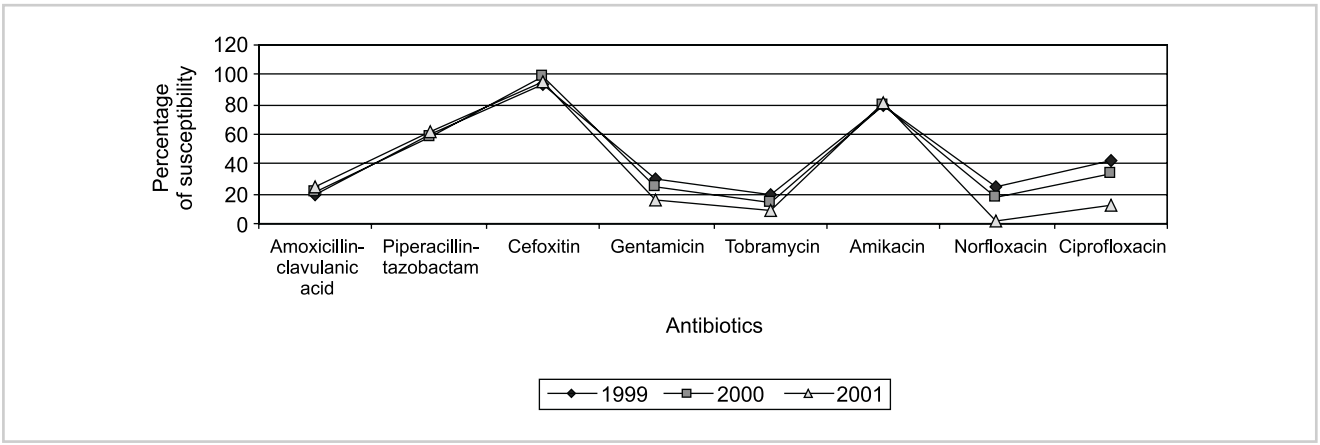

Figure 1. Susceptibility patterns of extended-spectrum betalactamase-producing strains of *E. coli.*

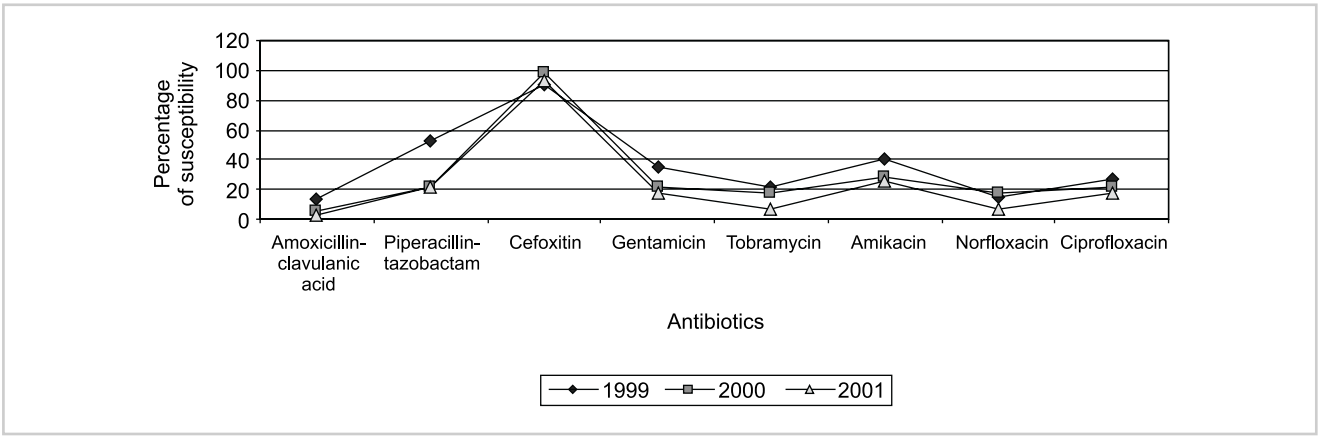

Figure 2. Susceptibility patterns of extended-spectrum betalactamase-producing isolates of *K. pneumoniae.*

tibility to all antibiotics was observed between 1999 and 2001, and no resistance to imipenem was detected.

As shown in Table 3, the isolates were distributed between the Intensive Care Unit (28.1% for *E. coli* and 34.8% for *K. pneumoniae*), medical wards, outpatients, and other origins. The highest numbers were found in the ICU. The isolates from outpatients increased in number every year.

Phenotypes of resistance

Based on the microbiological results with regard to the resistance of the ESBL-producing strains to ceftazidime and cefotaxime (not the interpreted results), three resistance phenotypes were observed (Table 4). Twelve *E. coli* isolates and 25 *K. pneumoniae* isolates were resistant to both antibiotics. While 19 *E. coli* and 20 *K. pneumoniae* isolates were resistant to ceftazidime and susceptible to cefotaxime, only five *E. coli* and two *K. pneumoniae* isolates were susceptible to ceftazidime and resistant to cefotaxime.

DISCUSSION

Although several studies have addressed the issue of the emergence of ESBL-producing strains of *E. coli* and *K. pneumoniae* worldwide (14-18), very few articles have addressed this issue in Lebanon (9). The published information indicates that the prevalence of ESBL among *E. coli* and *K. pneumoniae* isolates was 3.3% and 6.4% respectively in 1999. These data, while different from our results for 1999 (1.2% and 19.7% respectively for both bacteria), stress the importance of establishing national surveillance and control programs to combat antimicrobial resistance in Lebanon.

The percentages of ESBL-producing strains among *K. pneumoniae* were higher than those among *E. coli.* The percentages of *E. coli* did not significantly fluctuate during the 5 years studied, although an increase was observed in 2000 and 2001 (2.3% and 4.0% respectively). A different pattern was recorded for *K. pneumoniae*: the prevalence of

Table 3. Origins of the extended-spectrum betalactamase (ESBL)-producing isolates of *E. coli* and *K. pneumoniae* over the 5-year study period.

	ICU	Hospital wards	Postoperative	Other origin (from hospital)	Outpatients	Total
ESBL-positive *E. coli* isolates						
1997	2	2	2	1	0	7
1998	3	0	3	1	0	7
1999	3	4	4	0	0	11
2000	13	6	4	2	3	28
2001	4	9	7	6	10	36
Total (%)	25 (28.1)	21(23.5)	20 (22.5)	10 (11.2)	13 (14.6)	89
ESBL-positive *K. pneumoniae* isolates						
1997	5	1	0	3	0	9
1998	12	9	8	7	2	38
1999	23	5	0	3	9	40
2000	35	26	19	12	18	110
2001	10	9	7	3	18	47
Total (%)	85 (34.8)	50 (20.5)	34 (13.9)	28 (11.5)	47 (19.3)	244

the resistant strains increased continuously and reached a maximum of 24% in 2000 and 2001. The highest number of ESBL-producing strains was isolated from urine specimens.

Hospital outbreaks of ESBL-producing *Enterobacteriaceae* have been observed at an increasing frequency in recent years and the strains have often been characterized by multiresistance (19). Our results confirm those of the studies cited above in that ESBL-producing strains appear to be resistant to other classes of antimicrobial drugs. Our data showed that these strains were highly resistant to the quinolones, aminoglycosides and betalactam-betalactamase inhibitor combinations. Amikacin showed the highest percentage of susceptibility among the aminoglycosides and ranked second after imipenem for all the antibiotics tested. A total of 49% or fewer of the ESBL-producing strains were susceptible to cefoxitin; as this antibiotic is not hydrolyzed by ESBLs, this rate of resistance to cefoxitin is relatively high.

Table 4. Phenotypes of susceptibility of extended-spectrum betalactamase (ESBL)-producing isolates of *E. coli* (36 isolates) and *K. pneumoniae* (47 isolates) in 2001.

ESBL-positive *E. coli*	ESBL-positive *K. pneumoniae*	Phenotypes of resistance	
		Ceftazidime	Cefotaxime
12 (33.3%)	25 (53.2%)	Resistant	Resistant
19 (52.8%)	20 (42.5%)	Resistant	Susceptible
5 (13.9%)	2 (4.3%)	Susceptible	Resistant

The quality control results showed that the antibiotic susceptibility testing methods were adequate. Due to the limited epidemiological data in Lebanon regarding ESBL-producing strains, a comparison with other institutions is not possible. Preliminary data in our hospital show that 83.7% of non-ESBL-producing strains of *E. coli* and 92% of non-ESBL-producing strains of *K. pneumoniae* are susceptible to cefoxitin.

It has been reported that the strains producing the enzymes SHV and TEM show more activity against ceftazidime than cefotaxime, whereas, CTX-M-producing strains are in general more active against cefotaxime than ceftazidime (4, 5, 19, 20). Although a confirmation of the genotype of these enzymes is essential, the resistance phenotypes can be considered important preliminary indicators. The high percentage of resistance to both ceftazidime and cefotaxime, compared with bacteria resistant to only one of the two antibiotics, illustrates the increasing levels of resistance to betalactams of clinically important bacteria. An extrapolation of these results would suggest that the occurrence of CTX-M enzymes alone is still low in our hospital isolates.

In conclusion, our study is an example of the increasing frequency of ESBL among clinical isolates, which may be due to the excessive use of broad-spectrum antibiotics. The clinical use of these antibiotics is becoming a challenge in our country due to the limited options when the infection is produced by ESBL-producing *E. coli* and *K. pneumoniae.* Therefore, antibiotic control policies must be considered mandatory in order to minimize antibiotic resistance. More-

over, the present study has been the first to assess the occurrence and susceptibility patterns of ESBL-producing *Enterobacteriaceae* in Lebanon. Epidemiological and molecular studies should be conducted to establish the relationship between ESBL-producing isolates and to characterize the different types of ESBL.

REFERENCES

1. Stobberingh, E.E., Arends, J., Hoogkamp-Korstanje, J.A.A. et al. *Occurrence of extended-spectrum beta-lactamases in Dutch hospitals*. Infection 1999; 27: 348-354.
2. Branger, C., Lesimple, A.L., Bruneu, B., Berry, P., Lambert-Zechovsky, N. *Long-term investigation of the clonal dissemination of Klebsiella pneumoniae isolates producing extended-spectrum β-lactamases in a university hospital.* J Med Microbiol 1998; 47: 201-209.
3. Bradford, P.A. *Extended-spectrum β-lactamases in the 21st century: Characterization, epidemiology, and detection of this important resistance threat*. Clin Microbiol Rev 2001; 14: 933-951.
4. Coudron, P.E., Molland, E.S., Sanders, C.C. *Occurrence and detection of extended specrum beta-lactamase in members of the family Enterobacteriacese at a veterans medical center: Seek and you may find.* J Clin Microbiol 1997; 35: 2593-2597.
5. Franceschini, N., Perilli, M., Segatore, B., Setacci, D., Amicosante, G. *Ceftazidime and aztreonam resistance in Providencia stuartii: Characterization of a natural TEM-derived ESBL. TEM-60.* Antimicrob Agents Chemother 1998; 42: 1459-1462.
6. Rice, L.B. *Successful interventions for gram-negative resistance to extended-spectrum β-lactam antibiotics*. Pharmacotheropy 1999; 19: 120S-128S.
7. Rice, L.B., Eckstein, E.C., DeVente, J., Shlaes, D.M. *Ceftazidime-resistant Klebsiella pneumoniae isolates recovered at the Cleveland Department of Veterans Affairs Medical Center.* Clin Infect Dis 1996; 23: 118-124.
8. Peña, C., Pujol, M., Ricart, A. et al. *Risk factors for faecal carriage of Klebsiella pneumoniae producing extended-spectrum β-lactamase in the intensive care unit.* J Hosp Infect 1997; 35: 9-16.
9. Araj, G.F. *Antimicrobial resistance in Lebanon.* APUA 1999; Newsletter 17: 1-4.
10. Farmer, J.J., III. *Enterobacteriaceae: Introduction and identification.* In: P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H. Yolken (Eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C. 1995; p. 438-449.
11. NCCLS. Performance Strandards for Antimicrobial Susceptibility Testing; Eighth International Supplement. NCCLS document M100-S8. NCCLS, Wayne, PA 1999.
12. Jarlier, V., Nicolas, M., Fournier, G., Philippon, A. *Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in Enterobacteriaceae: Hospital prevalence and susceptibility patterns.* Rev Infect Dis 1988; 10: 867-878.
13. National Committee for Clinical Laboratory Standards. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A5 and informational supplement M100-S10.* National Committee for Clinical Laboratory Standards, Wayne, PA 2000.
14. Arlet, G., Sanson-Le Pors, M.J., Rouveau, M. et al. *Outbreak of nosocomial infections due to Klebsiella pneumoniae producing SHV-4 β-lactamase.* Eur J Clin Microbiol Infect Dis 1990; 9: 797-803.
15. Bauerfeind, A., Rosenthal, E., Eberlein, E., Holley, M., Schweighart, S. *Spread of Klebsiella pneumoniae producing SHV-5 β-lactamase among hospitalized patients.* Infection 1993; 21: 18-22.
16. Brun-Buisson, C., Legrand, P., Philippon, A., Montravers, F., Ansquer, M., Dural, J. *Transferable enzymatic resistance to third-generation cephalosporins during nosocomial outbreak of multiresistant Klebsiella pneumoniae.* Lancet 1987; ii: 302-306.
17. Brun-Buisson, C., Legrand, P., Philippon, A., Montravers, F., Ansquer, M., Dural, J. *Transferable enzymatic resistance to third-generation cephalosporins during nosocomial outbreak of multiresistant Klebsiella pneumoniae.* Lancet 1987; ii: 302-306.
18. Johnson, A.P., Weinbren, M.J., Ayling-Smith, B., Du Bois, S.K., Amyes, S.G.B., George, R.C. *Outbreak of infection in two UK hospitals caused by a strain of Klebsiella pneumoniae resistant to cefotaxime and ceftazidime.* J Hosp Infect 1992; 20: 97-103.
19. Rahal, J.J. *Extended-spectrum- beta-lactamases: How big is the problem?* Clin Microbiol Infect 2000; 6 (Suppl. 2): 2-6.
20. Livermore, D.M. *Beta-lactamases in laboratory and clinical resistance.* Clin Mic Rev Oct 1995; 8: 4: 557-584.