EPIDEMIOLOGY OF EXTENDED-SPECTRUM BETA-LACTAMASE PRODUCING GRAM-NEGATIVE BACILLI AT SIRIRAJ HOSPITAL, THAILAND, 2003

Methee Chayakulkeeree[1], Pichai Junsriwong[1], Anuwat Keerasuntonpong[1], Chanwit Tribuddharat[2] and Visanu Thamlikitkul[1]

[1]Department of Medicine, [2]Department of Microbiology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand

Abstract. A cross-sectional study was conducted from August to September, 2003 to determine the prevalence and risk factors in acquiring extended-spectrum beta-lactamase (ESBL) producing gram-negative bacilli (GNB) in patients admitted to Siriraj Hospital and the outcomes of these infections. Of 346 isolates of gram-negative bacteria in 249 patients, 102 isolates from 87 patients were colonization only, but 244 isolates from 162 patients were infections. The common GNB were *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Enterobacter cloacae*. The overall prevalence of ESBL producers was 30.1%. *K. pneumoniae* had a very high prevalence of ESBL producers (56.9%). The urinary tract was the most common site for ESBL- producing GNB infections. Nosocomial infections, duration from admission to infection, peripheral line, urinary catheterization, nasogastric tube insertion and previous use of beta-lactams, cephalosporins and fluoroquinolones were associated with acquiring ESBL-producing GNB infections. ESBL-producing GNB were significantly more resistant to antimicrobial agents. More than 80% of ESBL-producing GNB were susceptible to carbapenems. Mortality in patients infected with ESBL-producing GNB (41.3%) was significantly higher than those infected with non- ESBL-producing GNB (19.8%).

INTRODUCTION

Bacteria can be resistant to antimicrobial agents by various mechanisms. Beta-lactamase production is the most important mechanism for bacterial resistance to beta-lactam antibiotics and many beta-lactamases have been identified. Since *Klebsiella pneumoniae* with ESBL production was first isolated from the University Hospital in Frankfurt, Germany in 1983 (Knothe *et al*, 1983), many outbreaks caused by multi-resistant strains have been reported all over the world. In Asia, ESBL-producing bacteria have been reported in China (Jacoby and Medeiros, 1991, Cheng and Chen, 1994), Japan (Yagi, *et al*, 2000), Singapore (Jacoby and Medeiros, 1991), Korea (Kim *et al*, 1998) and Thailand (Chanawong *et al*, 2001). In Thailand, Asawapokee *et al* (1994) reported that the prevalence of ESBL-producers in *K. pneumoniae* was 39% in 1994 and in *E. coli* 10% in 1996.

ESBLs are plasmid-mediated enzymes that hydrolyze broad-spectrum beta-lactams and are strongly inhibited by clavulanate. ESBLs are transmitted by plasmids among bacteria and difficult to detect by routine antimicrobial susceptibility tests. Furthermore, antibiotics such as trimethoprim-sulfamethoxazole, aminoglycosides and fluoroquinolones (Martinez-Martinez *et al*, 1998) are often co-transferred on a resistance plasmid, resulting in multiple drug resistance. Thus clinical treatment failure occurs frequently, especially when inappropriate antimicrobial therapy is used to treat infections caused by ESBL- producing organisms. Therefore, if infections with ESBL-producing organisms can be predicted by the clinical characteristics of patients, this may lead to a better selection of antibiotics and may improve the outcome of infections.

The objectives of this study were to deter-

Correspondence: Visanu Thamlikitkul, Department of Medicine, Siriraj Hospital, 2 Prannok Road, Bangkok 10700, Thailand.
Tel: 66 (0) 2412 5994
E-mail: sivth@mahidol.ac.th

mine the prevalence of ESBL-producing GNB, the risk factors for infections with ESBL-producing GNB, antibiotic susceptibility patterns of ESBL-producing GNB, and the outcomes of patients infected with ESBL-producing GNB.

MATERIALS AND METHODS

The study protocol was approved by the Ethics Committee on Human Research at Siriraj Hospital. From August to September 2003, all isolates of GNB from clinical specimens collected from patients admitted to Siriraj Hospital were identified and tested for the existence of ESBL as well as their susceptibility to antibiotics. ESBL was determined according to the criteria of the National Committee for Clinical Laboratory Standards (2000) by means of a disk diffusion method. A combination disk diffusion method was performed using a ceftazidime disc (30 µg) and a ceftazidime/clavulanate disk (30/10 µg) placed onto Muller-Hinton agar containing GNB and incubated for 24 hours. ESBL production was indicated if the diameter of the inhibition zone around the ceftazidime/clavulanate disk was $\geq$5 mm greater than the diameter of the inhibition zone around the ceftazidime disk. All patients older than 15 years, from whom GNBs were isolated, were prospectively followed. All relevant information on demographics, risk factors for infection, type of infection, antibiotic therapy and outcomes, were collected from the patients and their medical records. Statistical analysis was performed using SPSS. Chi-square, Fisher's exact test, and Student's *t*-test were used where appropriate. All statistical tests were two-sided, and $p<0.05$ was considered significant.

RESULTS

During the study period, 346 isolates of GNB from 249 patients were suitable for analysis. Of the 346 isolates of GNB, 244 isolates from 162 patients were infections, 102 isolates from 87 patients were colonizations. The prevalence of ESBL-producing GNB is shown in Table 1. The overall prevalence of ESBL producers was 30.1%. *K. pneumoniae* had a very high prevalence of ESBL production (56.9%). Of the 162 patients who had GNB infections, 31.5 % were infected with ESBL-producing GNB and 68.5% were infected with non-ESBL-producing GNB. The characteristics of the patients infected with ESBL-producing GNB and non-ESBL-producing GNB are shown in Table 2. The characteristics

Table 1
Prevalence of ESBL producers in 346 isolates of GNB.

Gram-negative bacilli	ESBL negative N (%)	ESBL positive N (%)	Total N (%)
Escherichia coli	70 (66.7)	35 (33.3)	105 (30.3)
Klebsiella pneumoniae	22 (43.1)	29 (56.9)	51 (14.7)
Pseudomonas aeruginosa	50 (79.4)	13 (20.6)	63 (18.2)
Nonfermentative gram-negative bacilli	23 (74.2)	8 (25.8)	31 (9.0)
Enterobacter cloacae	13 (54.2)	11 (45.8)	24 (6.9)
Salmonella spp	2 (66.6.0)	1 (33.3)	3 (0.9)
Aeromonas hydrophila	1 (100.0)	0 (0.0)	1 (0.3)
Acinetobacter baumannii	41 (93.2)	3 (6.8)	44 (12.7)
Haemophilus influenzae	2 (100.0)	0 (0.0)	2 (0.6)
Serratia marcescens	3 (100.0)	0 (0.0)	3 (0.9)
Providentia stuatii	0 (0.0)	1 (100.0)	1 (0.3)
Morganella morganii	0 (0.0)	2 (100.0)	2 (0.6)
Vibrio spp	1 (100.0)	0 (0.0)	1 (0.3)
Proteus mirabilis	14 (93.3)	1 (6.7)	15 (4.3)
Total	242 (69.9)	104 (30.1)	346 (100.0)

Table 2
Characteristics of the patients infected with ESBL-producing GNB and non-ESBL-producing GNB.

Characteristic	ESBL		p-value
	Negative 111 patients	Positive 51 patients	
Male	58 (52.3%)	25 (49.0%)	0.83
Mean age (SD), years	59.5 (18.8)	60.9 (20.4)	0.68
Type of ward			
General ward	69 (62.2%)	37 (72.5%)	0.44
Private ward	36 (32.4%)	12 (23.5%)	
ICU	6 (5.4%)	2 (3.9%)	
Department			
Medicine	59 (53.2%)	29 (56.9%)	0.79
Other departments	52 (46.8%)	22 (43.1%)	
Underlying diseases			
Diabetes mellitus	31 (27.9%)	12 (23.5%)	0.69
Hypertension	36 (32.4%)	11 (21.6%)	0.22
Heart diseases	20 (18.0%)	8 (15.7%)	0.89
COPD	9 (8.1%)	3 (5.9%)	0.75
Chronic renal failure	8 (7.2%)	5 (9.8%)	0.55
Chronic liver disease	4 (3.6%)	4 (7.8%)	0.26
Cerebrovascular disease	19 (17.1%)	9 (17.6%)	1.00
Malignancy	33 (29.7%)	21 (41.2%)	0.21
Neutropenia	6 (5.4%)	2 (3.9%)	1.00
HIV infection	5 (4.5%)	0	0.33
Others	100 (90.1%)	50 (98.0%)	0.11
Site of infections			
Respiratory tract	52 (46.8%)	23 (45.1%)	1.00
Urinary tract	65 (58.6%)	36 (70.6%)	0.26
Skin and soft tissue infection	20 (18.01%)	8 (15.7%)	0.92
Gastrointestinal tract	16 (14.4%)	6 (11.8%)	0.87
CNS infection	1 (1%)	0	1.00
Bone and joint infection	3 (2.7%)	0	0.56
Primary bacteremia	11 (9.9%)	3 (5.9%)	0.56
Nosocomial infections	60 (54.1%)	39 (76.5%)	0.01
Median duration from admission to infection (interquartile range), days	4 (13)	13 (25)	0.01
Kanofky performance status score (median)	60	50	<0.001
Presence of central intravenous line	12 (10.8%)	7 (13.7%)	0.79
Presence of peripheral venous line	67 (60.4%)	43 (84.3%)	0.004
Parenteral nutrition	4 (3.6%)	2 (3.9%)	1.00
Mechanical ventilation	23 (20.7%)	15 (29.4%)	0.31
Endotracheal intubation	23 (20.7%)	15 (29.4%)	0.31
Tracheostomy	7 (6.3%)	4 (7.8%)	0.74
Urinary catheterization	54 (48.6%)	37 (72.5%)	0.01
Nasogastric tube insertion	33 (29.7%)	28 (54.9%)	0.004
Gastrostomy/jejunostomy	2 (1.8%)	0	1.00
Peritoneal dialysis	3 (2.7%)	4 (7.8%)	0.21
Hemodialysis	2 (1.8%)	2 (3.9%)	0.59
Decubitus ulcer	8 (7.2%)	8 (15.7%)	0.16
Chemotherapy	5 (4.5%)	4 (7.8%)	0.46
Immunosuppressive agents	17 (15.3%)	7 (13.7%)	0.98
Other invasive procedures/surgery	25 (22.5%)	17 (33.3%)	0.21
Previous antibiotic treatment	36 (32.4%)	32 (62.7%)	0.001
Previous beta-lactam treatment	28 (25.2%)	26 (51.0%)	0.002
Previous cephalosporin treatment	20 (18.0%)	21 (41.2%)	0.003
Previous carbapenem treatment	6 (5.4%)	6 (11.8%)	0.2
Previous aminoglycoside treatment	7 (6.3%)	7 (13.7%)	0.19
Previous fluoroquinolone treatment	5 (4.5%)	12 (23.5%)	0.001

Table 3
Antibiotic susceptibility patterns of ESBL-producing GNB and non-ESBL-producing GNB.

Antibiotic	ESBL-negative Number susceptible/ total tested isolates (%)	ESBL-positive Number susceptible/ total tested isolates (%)	p-value
Ampicillin	25/197 (12.7%)	1/90 (1.1%)	0.003
Cefazolin	96/195 (49.2%)	2/88 (2.3%)	<0.001
Amikacin	182/241 (75.5%)	64/103 (62.1%)	0.017
Gentamicin	150/241 (62.2%)	29/103 (28.2%)	<0.001
Cotrimoxazole	73/242 (30.2%)	17/102 (16.7%)	0.014
Amoxicillin/clavulanate	81/199 (40.7%)	3/93 (3.2%)	<0.001
Ampicillin/sulbactam	91/199 (45.7%)	8/91 (8.8%)	<0.001
Cefotaxime	122/242 (50.4%)	2/103 (1.9%)	<0.001
Ceftriaxone	122/242 (50.4%)	2/103 (1.9%)	<0.001
Ceftazidime	179/242 (74.0%)	5/104 (4.8%)	<0.001
Sulbactam/cefoperazone	180/239 (75.3%)	44/104 (42.3%)	<0.001
Norfloxacin	35/80 (43.8%)	2/30 (6.7%)	0.001
Ciprofloxacin	123/240 (51.3%)	19/104 (18.3%)	<0.001
Cefpirome	173/237 (73%)	26/104 (25.0%)	<0.001
Cefepime	186/240 (77.5%)	50/104 (48.1%)	0.006
Imipenem	185/240 (77.1%)	94/104 (90.4%)	0.019
Meropenem	181/237 (76.4%)	90/102 (88.2%)	<0.001
Tazobactam/piperacillin	182/240 (75.8%)	54/104 (51.9%)	<0.001

found to be significantly associated with the acquisition of ESBL-producing organisms were nosocomial infections, duration of hospitalization prior to infections, presence of peripheral intravenous catheter, insertion of urinary catheter, insertion of a nasogastric tube, prior use of beta-lactams, cephalosporins or fluoroquinolones within the previous 3 months, and lower Karnofsky performance status score. The antibiotic susceptibility patterns for ESBL-producing GNB and non-ESBL-producing GNB are shown in Table 3. ESBL-producing GNB were significantly more resistant to antimicrobial agents except for the carbapenems. About 90% of ESBL-producing GNB were susceptible to carbapenems compared with 77% in non-ESBL-producing GNB. The mortality rate in patients infected with ESBL-producing organisms was 43.1% compared to 19.8% in patients with non-ESBL-producing GNB (p=0.008).

DISCUSSION

Earlier studies on the prevalence of ESBL-producing organisms in eight medical centers in Thailand showed that the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* during 1996-1999 was 15.7% and 45.6%, respectively (Biedenbach *et al*, 1999). The annual reports of ESBL-producing organisms at another university hospital in Thailand revealed that the prevalence of ESBL-producing *K. pneumoniae* increased from 10.2% in 1998 to 46.5% in 2001. The present study observed that an overall prevalence of ESBL-producing gram-negative bacteria was 30.1% and the prevalence of ESBL-producers in *E. coli*, *K. pneumoniae* and *Enterobacter cloacae* increased to 33.3%, 56.9% and 45.8%, respectively. The prevalence of ESBL-producing *E. coli* and *K. pneumoniae* in our study was higher than in previous studies from the same institution and different institutions in Thailand. This observation indicated that ESBL production has played an important role in the resistance mechanism of GNB in Thailand, especially in tertiary care institutions. Previous studies suggested that urinary catheters, nasogastric tubes, central venous catheters, arterial cath-

eters, endotracheal tubes, ventilators, total parenteral nutrition and emergency abdominal surgery were related to colonization or infection with ESBL-producing organisms (Lautenbach *et al*, 2001; Nathisuwan *et al*, 2001; Ho *et al*, 2002; Kang *et al*, 2004a,b). Our study found that the factors significantly associated with the acquisition of ESBL-producing GNB were nosocomial infections, longer durations of hospitalization prior to infection, presence of peripheral intravenous catheters, urinary catheters, nasogastric tubes, prior uses of beta-lactams, cephalosporins or fluoroquinolones within the previous 3 months and a lower Kanofsky performance status score. Our study did not observe any correlation between co-morbid conditions of the patient and the acquisition of organisms with ESBL-production as reported by others (Lin *et al*, 2003). Our study showed that the Karnofsky performance status score was lower in the group with ESBL-producing GNB infections. One study also showed that patients with fecal colonization of ESBL-producing *K. pneumoniae* had a higher clinical severity score on admission (Pena *et al*, 1997). We found that nosocomial infection was a significant risk factor and the longer the patients were hospitalized, the more likely they were to be colonized and infected with ESBL-producing GNB. Paterson *et al* (1997) found that 31% of patients with ESBL-producing *K. pneumoniae* had received a third generation cephalosporin within the 14 days preceding bacteremia compared with 3% in the control group. The widespread use of third generation cephalosporins is believed to be the major cause of the mutation in these enzymes that have led to the emergence of ESBL-producing bacteria (Rice *et al*, 1990; Naumovski *et al*, 1992). An association between fluoroquinolones and the emergence of ESBL production has also been observed (Paterson *et al*, 2000). A possible explanation for the co-existence of these two resistance mechanisms is that they were transferred on the same plasmid. The study of plasmid-mediated ciprofloxacin resistance has recently been reported (Martinez-Martinez *et al*, 1998).

In our study, prior use of beta-lactams or fluoroquinolones was associated with infections caused by ESBL-producing GNB. Prior use of aminoglycosides was also found more often in patients infected with ESBL-producing GNB, though it did not reach a significant level due to the small sample size. Our findings confirmed an association between antimicrobial use and the emergence of ESBL-producing GNB. The ESBL-producing GNB in our series was significantly less susceptible to all antimicrobial agents when compared with non- ESBL-producing GNB, except for carbapenems. This may be explained by the fact that ESBL was not detected in most strains of *Acinetobacter baumannii* (93%), and the prevalence of carbapenem resistant *Acinetobacter baumannii* was high. Of 55 strains which were ESBL-negative and carbapenem-resistant, 12 were *Pseudomonas aeruginosa*, 8 were non-fermenters and 35 were *Acinetobacter baumannii*. Carbapenem is the most active and reliable treatment regimen for infections caused by ESBL-producing GNB (Paterson *et al*, 2004a,b; Kang *et al*, 2004a,b). Mortality in patients infected with ESBL-producing GNB was high (41.3%), even in patients who received carbapenems. This observation can be explained by a high prevalence of imipenem-resistance (5 of 16 strains) and delayed treatment (3 patients received carbapenem treatment 72 hours after the onset of infection). Therefore, infections caused by ESBL-producing GNB have become more important in Thailand due to limited effective antibiotics and high mortality. Pena *et al* (1998) reported a significant role in rigorous restriction of oxyimino-â-lactam use in the management and successful control of a large nosocomial ESBL-producing *K. pneumoniae* outbreak. The New York Hospital also found a decrease in the rate of ESBL-producing *K. pneumoniae* upon a shift in antibiotic utilization from cephalosporins to other antibiotics (Rahal *et al*, 1998). However, with the limitation of healthcare costs, along with a limited number of broad spectrum antibiotics, cephalosporins remain the most common empiric antibiotics for Thai patients. The results from our study may be useful for selecting empiric antibiotics active against ESBL-producing GNB if the patient has the aforementioned factors associated with infection caused by ESBL-producing GNB.

ACKNOWLEDGEMENTS

The authors would like to thank Mr Suthipol Udompunthurak for his statistical analysis and The Thailand Research Fund for supporting the study. Dr Visanu Thamlikitkul is a recipient of the Senior Researcher Scholarship of the Thailand Research Fund.

REFERENCES

Aswapokee N, Pruksachatvuthi S, Charoensook B. Prevalence and susceptibility patterns of bacteria producing extended-spectrum beta-lactamases in a university hospital. *J Infect Dis Antimicrob Agents* 1994; 11: 49-53.

Biedenbach DJ, Johnson DM, Jones RN. In vitro evaluation of cefepime and other broad-spectrum beta-lactams in eight medical centers in Thailand. The Thailand Antimicrobial Resistance Study Group. *Diagn Microbiol Infect Dis* 1999; 35: 325-31.

Chanawong A, M'Zali FH, Heritage J, Lulitanond A, Hawkey PM. SHV-12, SHV-5, SHV-2a and VEB-1 extended-spectrum beta-lactamases in Gram-negative bacteria isolated in a university hospital in Thailand. *J Antimicrob Chemother* 2001; 48: 839-52.

Cheng Y, Chen M. Extended-spectrum beta-lactamases in clinical isolates of *Enterobacter gergoviae* and *Escherichia coli* in China. *Antimicrob Agents Chemother* 1994; 38: 2838-42.

Ho PL, Chan WM, Tsang KW, Wong SS, Young K. Bacteremia caused by *Escherichia coli* producing extended-spectrum beta-lactamase: a case-control study of risk factors and outcomes. *Scand J Infect Dis* 2002; 34: 567-73.

Jacoby GA, Medeiros AA. More extended-spectrum beta-lactamases. *Antimicrob Agents Chemother* 1991; 35: 1697-704.

Kang CI, Kim SH, Park WB, *et al*. Bloodstream infections due to extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for mortality and treatment outcome, with special emphasis on antimicrobial therapy. *Antimicrob Agents Chemother* 2004a; 48: 4574-81.

Kang CI, Kim SH, Kim DM, *et al*. Risk factors for and clinical outcomes of bloodstream infections caused by extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae*. *Infect Control Hosp Epidemiol* 2004b; 25: 860-7.

Kim J, Kwon Y, Pai H, Kim JW, Cho DT. Survey of *Klebsiella pneumoniae* strains producing extended-spectrum beta-lactamases: prevalence of SHV-12 and SHV-2a in Korea. *J Clin Microbiol* 1998; 36: 1446-9.

Knothe H, Shah P, Krcmery V, Antal M, Mitsuhashi S. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. *Infection* 1983;11: 315-7.

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

Lin MF, Huang ML, Lai SH. Risk factors in the acquisition of extended-spectrum beta-lactamase *Klebsiella pneumoniae*: a case-control study in a district teaching hospital in Taiwan. *J Hosp Infect* 2003; 53: 39-45.

Martinez-Martinez L, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. *Lancet* 1998; 351: 797-9.

Naumovski L, Quinn JP, Miyashiro D, *et al*. Outbreak of ceftazidime resistance due to a novel extended-spectrum beta-lactamase in isolates from cancer patients. *Antimicrob Agents Chemother* 1992; 36: 1991-6.

National Committee for Clinical Laboratory Standards. Performance standard for antimicrobial disk susceptibility tests. Approved standard M7-A5. Wayne, Pa: National Committee for Clinical Laboratory Standards, 2000.

Nathisuwan S, Burgess DS, Lewis JS, 2nd. Extended-spectrum beta-lactamases: epidemiology, detection, and treatment. *Pharmacotherapy* 2001; 21: 920-8.

Paterson DL, Ko WC, Mohapatra S, *et al*. *Klebsiella pneumoniae* bacteremia: impact of extended spectrum beta-lactamase (ESBL) production in a global study of 216 patients [Abstract]. Presented at Toronto, Ontario, Canada: 37th Interscience Conference on Antimicrobial Agents and Chemotherapy; September 28 to October 1, 1997.

Paterson DL, Mulazimoglu L, Casellas JM, *et al*. Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum beta-lactamase production in *Klebsiella pneumoniae* isolates causing bacteremia. *Clin Infect Dis* 2000; 30: 473-8.

Paterson DL, Ko WC, Von Gottberg A, *et al*. Antibiotic therapy for *Klebsiella pneumoniae* bacteremia: implications of production of extended-spectrum beta-lactamases. *Clin Infect Dis* 2004a; 39: 31-7.

Paterson DL, Ko WC, Von Gottberg A, *et al*. International prospective study of *Klebsiella pneumoniae* bacteremia: implications of extended-spectrum beta-lactamase production in nosocomial Infections. *Ann Intern Med* 2004b; 140: 26-32.

Pena C, Pujol M, Ricart A, *et al*. Risk factors for faecal carriage of *Klebsiella pneumoniae* producing extended spectrum beta-lactamase (ESBL-KP) in the intensive care unit. *J Hosp Infect* 1997; 35: 9-16.

Pena C, Pujol M, Ardanuy C, *et al*. Epidemiology and successful control of a large outbreak due to *Klebsiella pneumoniae* producing extended-spectrum beta-lactamases. *Antimicrob Agents Chemother* 1998; 42: 53-8.

Rahal JJ, Urban C, Horn D, *et al*. Class restriction of cephalosporin use to control total cephalosporin resistance in nosocomial Klebsiella. *JAMA* 1998; 280: 1233-7.

Rice LB, Willey SH, Papanicolaou GA, *et al*. Outbreak of ceftazidime resistance caused by extended-spectrum beta-lactamases at a Massachusetts chronic-care facility. *Antimicrob Agents Chemother* 1990; 34: 2193-9.

Yagi T, Kurokawa H, Shibata N, Shibayama K, Arakawa Y. A preliminary survey of extended-spectrum beta-lactamases (ESBLs) in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* in Japan. *FEMS Microbiol Lett* 2000; 184: 53-6.