Original

**Analysis of TEM-type Genes Derived from
Cefotaxime-resistant *Serratia marcescens***

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Abstract: We investigated the TEM-type extended spectrum beta-lactamases (ESBLs) in cefotaxime-resistant *S. marcescens* strains by means of a 24-month retrospective study between October 1996 and September 1998 at Showa University Hospital. Among the 579 *S. marcescens* strains isolated during this period, 51 were resistant to cefotaxime. We tested the expression of ESBLs in the cefotaxime-resistant strains using the disk susceptibility test with cefotaxime and cefotaxime/clavulanic acid. We then amplified the beta-lactamase gene in each strain by the polymerase chain reaction (PCR).

The inhibition diameters observed with cefotaxime and cefotaxime plus clavulanic acid were similar in all of the resistant strains, suggesting that the cefotaxime-resistant *S. marcescens* strains do not express ESBLs. Sequence analysis of the PCR-amplified beta-lactamase gene from clinical isolates of the cefotaxime-resistant strains identified it as TEM-1b. We detected the TEM-type beta-lactamase genes, TEM-1a and TEM-1b, in four cefotaxime-sensitive strains. In the TEM-1a-like gene of a cefotaxime-sensitive strain, we found one base substitution at nucleotide position 753 (C to A) causing an amino acid substitution from alanine 184 to valine. No ESBL TEM enzymes were found among these strains, but we isolated a novel TEM-type enzyme.

The cefotaxime-resistant *S. marcescens* isolates, like many gram-negative enterobacteria, had the TEM-1b gene of beta-lactamase. Resistance to cefotaxime seemed to have been acquired through other ESBLs, cephalosporinases or carbapenemases, and not through TEM-type ESBLs.

Key words: TEM, ESBLs, penicillinases, *Serratia marcescens*, cefotaxime

Introduction

Class A beta-lactamases such as TEM-type beta-lactamases, which are penicillinases transferred by plasmids, have been detected in enterobacteria since the 1960's¹⁾. The excessive use of third-generation cephalosporins since the 1980's, mainly in Europe, has led to the development of extended-spectrum beta-lactamases (ESBLs) derived from class A beta-lactamases²⁾. Novel ESBLs have since developed worldwide including in Japan. These enzymes are class A beta-lactamases according to the Bush-Jacoby-Mederiros classification,

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and are defined as being inhibited by clavulanic acid³⁾. The relative hydrolytic efficacy of an ESBL should exceed 10% against cefotaxime, ceftazidime or aztreonam, assuming that the ESBL has 100% efficacy against penicillin G³⁾. In many countries including France, the U.S. and Italy, strains of the enterobacterium *Serratia marcescens* (*S. marcescens*) that produce ESBLs have recently appeared²⁾.

We have investigated *S. marcescens* strains that are resistant to antibiotics in patients at our hospital. Here, we describe the results of genetic analyses of *S. marcescens* strains that are resistant to third-generation cephalosporins, to determine whether or not the resistance was derived from TEM-type ESBLs.

Materials and Methods

Bacterial strains and culture

Fifty-one clinical isolates of cefotaxime-resistant *S. marcescens* strains were analyzed. These strains were identified from the 579 strains of *S. marcescens* isolated from patients seen at Showa University Hospital between October 1996 and September 1998. A *S. marcescens* strain was considered to be resistant to cefotaxime as measured by the sensitivity test using MIC 2000 (Dynatec, Virginia, USA)⁴⁾. Each strain was derived from a different patient.

To confirm whether each *S. marcescens* strain produced ESBLs, we followed the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (M100-S9): an ESBL-producing strain was identified by the disk method using the KB disk (Eiken Kagaku, Tokyo, Japan) with 30 µg cefotaxime or 30 µg/10 µg cefotaxime/clavulanic acid on Mueller Hinton S agar medium (Eiken Kagaku, Tokyo, Japan); a strain showing an inhibition circle whose diameter was at least 5 mm larger after the addition of clavulanic acid, was considered to be an ESBL-producing strain⁵⁾.

Polymerase chain reaction (PCR)

Three sets of original primers that recognize the common sequence of the TEM-type beta-lactamase gene, were synthesized to amplify nearly the entire TEM-type beta-lactamase gene.

1. Forward primer-1: 5'-GATCTCAACAGCGGTAAGATCC-3' Reverse primer-1: 5'-TCACTCATGGTTATGGCAGC-3' (Nucleotide positions 350-594, PCR product 254 bp)

2. Forward primer-2: 5'-TATGCAGTGCTGCCATAACC-3' Reverse primer-2: 5'-CGT-TCATCCATAGTTGCCTG-3' (Nucleotide positions 567-1020, PCR product 454 bp)

3. Forward primer-3: 5'-TATGCAGTGCTGCCATAACC-3' Reverse primer-3: 5'-CCA-GTGCTGCAATGATACC-3' (Nucleotide positions 567-950, PCR product 384 bp)

DNA from the clinically isolated bacteria was extracted using SepaGene (Sanko Junyaku, Tokyo, Japan). The PCR reaction (total 25 µl) was performed with the Gene Amp PCR System 9600-R (Perkin-Elmer, Norwalk, CT, USA). The PCR reaction mixture contained 100 ng of template DNA, 20 pmol of each primer, 200 µmol of each dNTP, 1 unit of Taq DNA polymerase (Roche, Basel, Switzerland), and PCR reaction buffer (+Mg) (Roche, Basel, Switzerland). The reaction conditions for primer set 1 were: 94°C for 30 sec, 60°C for 90 sec, 72°C for 90 sec, for 30 cycles. The reaction conditions for each of primer sets 2 and 3 were 94°C for 60 sec, 60°C for 60 sec, and 72°C for 120 sec, for 30 cycles. The PCR product was separated by polyacrylamide gel electrophoresis (PAGE) on a 5 %

polyacrylamide gel.

DNA sequencing

PCR products were cloned into the pGEM-T Easy™ vector using the TA cloning method according to the manufacturer's instructions (Promega, Madison, WI, USA). The cloning site is located inside the beta-galactosidase coding region. After transfection into JM109, the insert DNA-containing pGEM vector-transfectants were isolated as white colonies on a Luria base agar plate containing X-gal (5-bromo-4-chloro-3-indolyl-beta-galactoside) and IPTG (isopropyl-beta-D-thiogalactopyranoside). The plasmid DNA was extracted from each clone by the alkaline sodium dodecyl sulfate (SDS) method⁶. This DNA was then digested with EcoRI, and the presence of the insert DNA was confirmed by 5 % PAGE. After linearization with Sall, the plasmid DNA was sequenced with the A.L.F. DNA Sequencer II (Amersham Pharmacia, Uppsala, Sweden) using a fluorescent-labeled M13 forward sequencing primer (F5'CGACGTTGTAAAACGACGACGCGCCAGT3') and the Thermo Sequenase Fluorescent Labeled Primer Cycle Sequencing kit (Amersham Pharmacia, Uppsala, Sweden) as described previously⁷.

The position of the sequence was estimated from the position of the ampicillin resistance gene (TEM-1) as described by Sutcliffe⁸.

Results

Cefotaxime-resistant *S. marcescens*

Fig. 1 shows the proportion of cefotaxime-resistant *S. marcescens* isolates identified from

Fig. 1. Percentage of cefotaxime-resistant isolates among *Serratia marcescens* strains isolated during indicated time periods between 1991 and 1999 at Showa University hospital. Black bars represent proportion of resistant strains isolated during the indicated time period. S, susceptible ; I, intermediate ; R, resistant.

the total number of *S. marcescens* strains isolated at Showa University Hospital during the indicated periods between July 1991 through 1999. The percentage of cefotaxime-resistant *S. marcescens* is approximately 25–35% for each time period without any statistically significant difference.

PCR analysis of the TEM-gene and inhibition by clavulanic acid

Between October 1996 and September 1998, cefotaxime-resistant *S. marcescens* strains had been isolated from 51 patients. The patient characteristics and the departments in which they were treated are shown in Table 1. The minimum inhibitory concentration (MIC) value measured by the serial dilution method against major antibiotics is also shown. Whether the TEM-type beta-lactamase gene in each strain could be amplified with primer set 1, 2 or 3, is also shown in Table 1. Based on the definition given by Bush *et al.*³⁾, that the resistance of ESBL-producing strains against third-generation cephalosporins is inhibited by clavulanic acid, the disk method was employed using the KB disk, which contains 30 µg cefotaxime or 30 µg/10 µg cefotaxime/clavulanic acid on Mueller Hinton S agar medium. The results of this test are also shown in Table 1.

There was no noteworthy feature among the cefotaxime-resistant strains except that they were mainly isolated from urine and respiratory samples. All of the strains showed resistance to ampicillin and cefotiam. All of the imipenem-resistant strains had been derived from urine samples. Primer sets 1, 2 and 3 could amplify DNA fragments of a TEM-type beta-lactamase gene in 30 of the 51 cefotaxime-resistant strains. This indicates that some cefotaxime-resistant strains have TEM-type genes. In the ESBL recognition test that we performed, none of the 51 strains completely satisfied the definition of the presence of an ESBL. However, in some strains there was enlargement of the inhibition circle by less than 5 mm^{3, 5)}.

Sequencing of the TEM-gene

As described above, PCR could amplify a TEM-type beta-lactamase gene in 30 of the 51 cefotaxime-resistant strains. Genetic analyses of the PCR products from these 30 strains revealed that all of the corresponding PCR products had the same sequence. The amplified products contained a C-to-T transition at nucleotide position 436 and a G-to-T transversion at position 604 compared with the sequence of the TEM-1a gene⁸⁾, and it was identical to the TEM-1b gene^{9, 10)} which encodes penicillinase. The TEM-type ESBL gene was not identified (Fig. 2). Amino acids 21, 39 and 42 are substituted in various TEM derivatives, but since PCR primer set 1 starts at nucleotide 350 (amino acid number 47), we could not detect mutations occurring in the first 46 amino acids. Therefore, our sequencing data is incomplete (Fig. 2).

TEM gene of four cefotaxime-susceptible strains

Based on the data from PCR and sequencing, a TEM-type ESBL does not seem to be involved in the development of resistance to cefotaxime. Considering that the TEM-type genes obtained from the resistant strains encoded penicillinase, we next analyzed four *S. marcescens* strains that were sensitive to cefotaxime using the same procedure described above.

PCR analyses performed under the same conditions revealed a TEM-type gene in two of

the four cefotaxime-sensitive strains (strains A and B), that could be amplified with primer sets 1 and 3 but not with primer set 2 (Table 1).

Novel sequence of a TEM gene in cefotaxime-sensitive strains

Genetic analysis of the PCR products from strain A that had been obtained with primer set 1, revealed a sequence that was identical to the TEM-1a gene in three of the four clones of strain A. The fourth clone had a sequence that was identical to the TEM-1b or 1c gene, in which there was a mutation at position 436, a C-to-T transition, compared with the sequence of the TEM-1a gene (Fig. 2). With primer set 3, seven out of 12 clones showed an identical sequence with the TEM-1a gene except for a C-to-T transition at position 753, resulting in an alanine-to-valine substitution at amino acid 184. The remaining five clones had the TEM-1b gene with a C-to-T transition at position 436 and G-to-T transversion at position 604. The PCR products obtained from strain B with primer sets 1 and 3 were identical to the TEM-1b gene (Fig. 2). The alanine-to-valine amino acid substitution at 184 was assumed to have no influence on protein structure, and was thought to represent a polymorphism rather than a mutation. The encoded protein would therefore function as a beta-lactamase. The MIC data are consistent with this assumption (Table 1).

Discussion

The number of *S. marcescens* strains isolated from patients seen at our hospital is increasing every year. Most *S. marcescens* strains are isolated from respiratory specimens, followed by urine specimens¹³. The number of cefotaxime-resistant *S. marcescens* strains is also increasing. Most of the cefotaxime-resistant *S. marcescens* were isolated from urine specimens. This finding seems to represent a feature of most antibiotics that are excreted from the urinary tract. Because the urinary tract is exposed to high concentrations of antibiotics, prophlogistic bacteria are likely to acquire resistance and remain after screening.

ESBLs that can hydrolyze third-generation cephalosporin and aztreonam are considered to acquire the ability to hydrolyze novel antibiotics by mutation of the TEM-1a, TEM-1b, TEM-1c or TEM-2 genes, which code for plasmid-mediated penicillinases. The resulting amino acid substitutions are accompanied by a conformational change at the substrate-binding site^{3, 11, 12}. Most of the cefotaxime-resistant *S. marcescens* strains of interest contained a TEM-type gene. However, this TEM-type gene was the TEM-1b gene that encodes TEM-1 beta-lactamase. TEM-1 is a beta-lactamase that is commonly isolated from *S. marcescens*¹⁴⁻¹⁶. The TEM-1 beta-lactamase analyzed here seemed to be plasmid-mediated penicillinase, which is frequently observed in gram-negative enterobacteria. Since this penicillinase did not hydrolyze third-generation cephalosporines, resistance to cefotaxime seems to be acquired by other genetic resistance systems and not by TEM-type ESBLs.

The resistance of the cefotaxime-resistant strains isolated from the patients in this study may have been derived from a carbapenemase such as IMP-1, which is a class B metallo beta-lactamase^{17, 18}. The IMP-1 gene is usually located on a plasmid, and IMP-1 confers resistance not only to carbapenem but also to a broad spectrum of beta-lactams including third-generation cephalosporins¹⁷⁻¹⁹. In fact, 11 of the 51 strains were resistant to imipenem, (MIC value of above 8 µg/ml)⁴, and these 11 strains showed strong and broad resistance to several beta-lactams including ceftazidime. PCR analysis using specific primers for the IMP-1 gene revealed that 8 of the 11 imipenem-resistant strains and 12 others contained the

Table 1. Clinical data of patients from whom 51 cefotaxime-resistant *S. marcescens* strains were isolated and antimicrobial susceptibilities of the isolates

Strain	Specimen	Ward	PCR products*			MICs ($\mu\text{g/ml}$) by microdilution method										ESBL confirma- tion test (disk method)	
			1	2	3	ABPC	PIPC	CTM	CTX	CAZ	CMZ	IPM	AZT	GM	OFLX		
1	Urine	Uro	+	+	+	>16	>64	>16	>32	>16	>32	>8	16	8	>4	—	—
2	Urine	Uro	+	+	+	>16	>64	>16	>32	>16	>32	>8	16	4	>4	—	—
3	Urine	Uro	+	+	+	>16	>64	>16	>32	>16	>32	>8	>16	8	>4	—	—
4	Urine	Uro	+	+	+	>16	>64	>16	>32	>16	>32	>8	>16	>8	>4	—	—
5	Urine	Uro	+	+	+	>16	>64	>16	>32	>16	>32	>8	>16	8	>4	—	—
6	Urine	Uro	+	+	+	>16	>64	>16	>32	>16	>32	>8	>16	2	>4	—	—
7	Urine	Uro	+	+	+	>16	>64	>16	>32	>16	>32	>8	>16	>8	2	—	—
8	Urine	Uro	+	+	+	>16	>64	>16	>32	>16	>32	>8	>16	4	>4	—	—
9	Urine	Int	+	+	+	>16	>64	>16	>32	>16	>32	>8	4	>8	>4	—	—
10	Urine	Uro	+	+	+	>16	>64	>16	>32	>16	>32	>8	>16	>8	>4	—	—
11	Urine	Uro	—	—	—	>16	>64	>16	>32	>16	>32	>8	<8	8	4	—	—
12	Urine	Uro	+	+	+	>16	>64	>16	>32	>16	>32	4	>16	4	>4	—	—
13	Sputum	Int	+	+	+	>16	>64	>16	>32	<2	32	<1	<8	2	2	—	—
14	Sputum	Int	+	+	+	>16	>64	>16	>32	<2	8	<1	<8	<1	4	—	—
15	Pus	Derm	+	+	+	>16	>64	>16	>32	<2	32	<1	<8	4	4	—	—
16	Sputum	Int	+	+	+	>16	>64	>16	>32	16	>32	2	>16	8	>4	—	—
17	Throat swab	Int	+	+	+	>16	>64	>16	>32	>16	16	<1	>16	<1	4	—	—
18	Urine	Brain	+	+	+	>16	>64	>16	>32	8	>32	<1	16	<1	>4	—	—
19	Urine	Brain	+	+	+	>16	>64	>16	>32	<2	16	<1	<8	2	>4	—	—
20	Bronchial tube	Int	+	+	+	>16	>64	>16	>32	>16	16	<1	>16	4	4	—	—
21	Wound swab	Int	+	+	+	>16	>64	>16	>32	8	>32	2	16	<1	4	—	+ 3 mm
22	Urine	Int	+	+	+	>16	>64	>16	>32	4	>32	<1	<8	2	2	—	—
23	IVH catheter	Int	+	+	+	>16	>64	>16	>32	<2	>32	<1	<8	<1	>4	—	—
24	Urine	Abd.s	+	+	+	>16	>64	>16	>32	<2	>32	2	<8	2	>4	—	—
25	Surgery wound	Int	+	+	+	>16	>64	>16	>32	>16	>32	4	>16	2	>4	—	—
26	Urine	Brain	+	+	+	>16	>64	>16	>32	4	32	<1	<8	2	>4	—	—
27	Urine	Orth	+	+	+	>16	>64	>16	>32	8	>32	2	16	<1	4	—	+ 3 mm
28	Urine	Uro	+	+	+	>16	>64	>16	>32	4	>32	<1	<8	<1	4	—	+ 3 mm
29	Urine	Uro	+	+	+	>16	>64	>16	>32	16	>32	<1	>16	2	>4	—	+ 2 mm

30	Urine	Uro	+	+	+	>16	>64	>16	>32	16	>32	4	>16	4	>4	+4 mm
31	Urine	Int	+	+	+	>16	>64	>16	>32	>16	>32	2	>16	<1	4	+3 mm
32	Urine	Uro	-	-	-	>16	>64	>16	>32	8	32	<1	>16	<1	2	-
33	Urine	Uro	-	-	-	>16	>64	>16	>32	8	>32	<1	<8	<1	2	-
34	Urine	Uro	-	-	-	>16	>64	>16	>32	8	>32	<1	16	2	2	-
35	Urine	Abd.s	-	-	-	>16	>64	>16	>32	>16	>32	2	>16	<1	4	-
36	Ascites	Abd.s	-	-	-	>16	>64	>16	>32	>16	>32	<1	>16	<1	2	-
37	Sputum	Int	-	-	-	>16	>64	>16	>32	<2	32	<1	>16	2	4	-
38	Bronchial tube	Int	-	-	-	>16	>64	>16	>32	<2	16	<1	<8	<1	>0.5	-
39	Urine	Int	-	-	-	>16	>64	>16	>32	>16	>32	2	>16	2	>4	-
40	Oronial dischar	Oro	-	-	-	>16	16	>16	>32	8	16	<1	<8	<1	<0.5	-
41	Urine	Int	-	-	-	>16	>64	>16	>32	<2	32	2	<8	<1	2	-
42	Urine	Uro	-	-	-	>16	>64	>16	>32	8	>32	2	16	2	4	-
43	Urine	Uro	-	-	-	>16	>64	>16	>32	16	>32	2	>16	2	4	-
44	Catheter	Chest.s	-	-	-	>16	>64	>16	>32	>16	>32	2	>16	<1	4	-
45	Urine	Uro	-	-	-	>16	>64	>16	>32	>16	>32	2	>16	<1	4	-
46	Urine	Uro	-	-	-	>16	>64	>16	>32	>16	>32	<1	<8	<1	4	-
47	Urine	Obs	-	-	-	>16	>64	>16	>32	<2	>32	<1	<8	2	4	-
48	Urine	Uro	-	-	-	>16	>64	>16	>32	16	>32	2	>16	2	>4	+3 mm
49	Urine	Uro	-	-	-	>16	>64	>16	>32	>16	>32	<1	>16	<1	4	+2 mm
50	Urine	Int	-	-	-	>16	32	>16	>32	8	8	<1	16	<1	4	-
51	Urine	Int	-	-	-	>16	>64	>16	>32	4	>32	<1	16	<1	2	+4 mm
A	IVH catheter	Brain	+	-	+	>16	<8	>16	<8	<2	<4	<1	<8	<1	<0.5	
B	Urine	Uro	+	-	+	>16	32	>16	<8	8	16	<1	<8	<1	>4	
C	Sputum	Ane	-	-	-	16	<8	>16	<8	<2	8	<1	<8	<1	<0.5	
D	Artery blood	Chest.s	-	-	-	>16	<8	>16	<8	<2	32	<1	<8	<1	<0.5	

Data from 4 patients with cefotaxime-sensitive *S. marcescens* strains are also shown.

*+, PCR product amplified using indicated primer set from this strain;
 -, PCR product could not be amplified using indicated primer set from this strain.

Uro, Urology Department; Int, Internal Medicine; Derm, Dermatology; Brain, Neurosurgery; Abdominal Surgery; Orth, Orthopedic Surgery; Oto, Otolaryngology; Chest.s, Chest Surgery; Obs, Obstetrics and Gynecology; Ane, Anesthesiology; ABPC, ampicillin; PIPC, piperacillin; CTM, cefotiam; CTX, cefotaxime; CAZ, ceftazidime; CMZ, cefmetazole; IPM, imipenem; AZT, aztreonam; GM, gentamicin; OFLX, ofloxacin; MIC, minimum inhibitory concentration; IVH, intravenous high calorie infusion.

Fig. 2. Mutations in TEM-type beta-lactamase genes and experimental data. Only nucleotide differences from the TEM-1 beta-lactamase and/or *bla*_{TEM-1a} gene sequences are listed. Hatched bars in the beta-lactamase coding region indicate the sequence encoding the beta-lactam binding site. Data are adapted from published reports⁸⁻¹²⁾.

IMP-1 gene²⁰⁾. This observation also supports our hypothesis that the IMP-1 gene influences the resistance of the strains analyzed here.

Other than IMP-1, many mechanisms for the hydrolysis of third-generation cephalosporins in *Enterobacteriaceae* including *S. marcescens*, have been identified. For example, class C beta-lactamase is encoded by the cephalosporinase gene (*ampC*) located on the *S. marcescens* chromosome²¹⁾. The degree of induction of beta-lactamase expression varies depending on the type of beta-lactam used: carbapenem and cephamycins are powerful inducers of beta-lactamase expression, while third-generation cephalosporins are weak inducers. Originally, beta-lactamase had no influence on third-generation cephalosporins. However, after a long period of usage of third-generation cephalosporins, a mutated strain that produces the enzyme appeared and proliferated^{22, 23)}. This third-generation cephalosporin-resistant strain is only susceptible to carbapenem, which is stable against class C beta-lactamase. Therefore, ESBL inhibition by clavulanate would be masked by the presence of the AmpC enzyme that is present in all strains of *Serratia*. Furthermore, a number of our strains probably also possessed the IMP-1 beta-lactamase, which would mask the results of ESBL screening tests.

Other forms of acquired third-generation cephalosporin resistance include the following: SHV-type ESBLs^{11,24)}, which constitute a class of ESBLs; non-TEM, non-SHV-type KIT-1²⁵⁾; CTX-M1²⁶⁾; CTX-M2²⁷⁾; TOHO-1²⁸⁾; and TOHO-2²⁹⁾. These and plasmid-derived cephalosporinase³⁰⁾ are assumed to be the mechanisms of resistance in most of the ESBL-producing strains isolated in Japan. Our clinically isolated strains may have been influenced by the resistance described above. Although we used cefotaxime-resistant strains in this study, these strains may not be appropriate to set the standard for the sensitivity to cefotaxime, because most ESBLs show a low MIC value towards cefotaxime⁴⁾ regardless of whether they show a high MIC score towards ceftazidime or aztreonam. This problem will be solved by using the ESBL Confirmation Panel (Dade Behring, CA, USA), which has recently become commercially available.

A TEM-1a-like gene with a C-to-T transition (184 alanine to valine) was detected in one of the four cefotaxime-susceptible strains. This mutation was not identical to any previously detected mutation site in various TEM-type ESBLs (Fig. 2). This one-base substitution was accompanied by an amino acid substitution (184 alanine to valine). However this strain was still susceptible to cefotaxime and the spectrum of susceptibility was not significantly different from that of frequently isolated *S. marcescens* strains. Taken together, we concluded that the TEM-1b gene isolated from the cefotaxime-resistant *S. marcescens* strains is not specific for these strains, but is commonly observed in *S. marcescens* strains.

The frequency with which cefotaxime-resistant *S. marcescens* is isolated at our hospital will likely should increase. This resistant property is not derived from TEM-type ESBLs since we detected the TEM-1b gene which produces TEM-1, a common penicillinase, in *S. marcescens* strains. In addition to the TEM-1b gene, which was also found in some of the sensitive strains, one strain carried a TEM-1a-like gene.

Few reports have described ESBL-producing strains in Japan, compared with European countries. To prevent an outbreak, the mechanism of resistance to beta-lactams must be elucidated, since these antibiotics play an important role in the therapy of infectious diseases.

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