Resistance-gene Analysis of Extended-spectrum β-lactamase-producing Bacteria in Our Clinic

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Objective

Extended-spectrum β-lactamases (ESBLs) are class A β-lactamases that degrade penicillin antibiotics, namely, enzymes that hydrolyze a wide spectrum of third- and fourth-generation antibiotics. Furthermore, most of these enzymes cause resistance to aminoglycoside and new quinolone antibiotics. The ESBL resistance genes are transmitted via plasmids across bacterial strains. Thus, we should be cautious regarding resistant bacteria to prevent hospital-acquired infection.

In our bacteriological laboratory, ESBL-producing bacteria are detected with the Microscan WalkAway 96SI microbiology system (Dade Behring, Deerfield, IL, USA), and the detection rate has been increasing every year. However, resistance genes cannot be analyzed any further with this method.

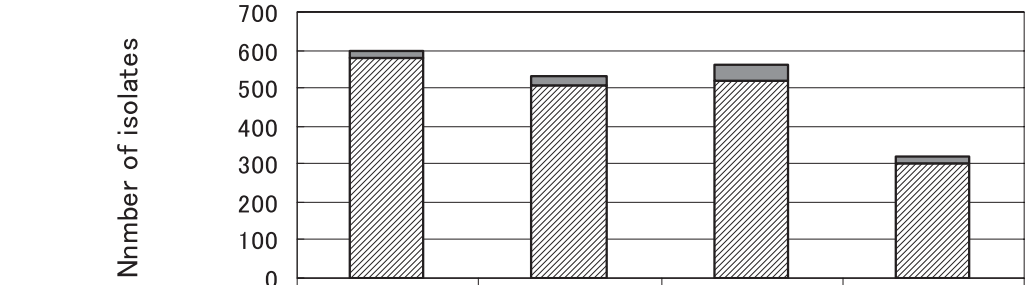
In the present study, we analyzed the CTX M-1, -2, -9, TEM, and SHV resistance genes using the polymerase chain reaction (PCR).

Subjects and Methods

Resistance genes were investigated with PCR and the primers specific to CTX M-1, -2, -9, TEM, and SHV for the 56 strains of *Escherichia coli* and 49 strains of *Klebsiella pneumoniae* isolated in our clinic from January 2008 through June 2009.

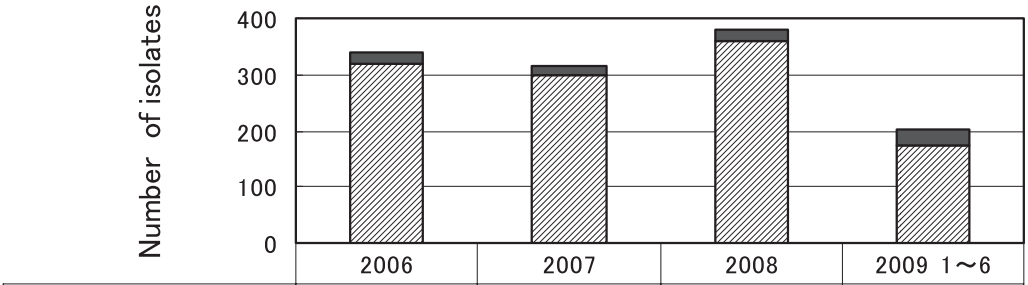
Results

The annual ESBL isolation status is shown in **Figures 1** and **2**. The detection rates of ESBL-producing bacteria are increasing for both *E. coli* and *K. pneumoniae*. Monthly isolation rates during the collection period of both ESBLs, used for analysis, are shown in **Figure 3**. In this period, the isolation frequency did not change for *E. coli* but increased for *K. pneumoniae*. The results of the analysis of ESBL resistance genes are shown in **Table 1**. CTX M-9 was predominant (53.6%) for *E. coli*, followed by CTX M-1 and -2. The TEM single expression rate was 16.1%. Its coexpression rate with CTX M was 58.9%. On the other hand, CTX M-1 was dominant (63.3%) for *K. pneumoniae*. Its coexpression rates with TEM and SHV were 73.4 and 55.1%, respectively.



	2006	2007	2008	2009 1～6
□ %	2.90%	4.50%	7.70%	7.40%
ESBL-producing *E.coli* strains	17	23	40	22
All *E.coli* strains	582	507	521	299

Fig. 1 Annual isolation of ESBL-producing *E. coli* strains



	2006	2007	2008	2009 1～6
□ %	5.90%	5.00%	5.60%	16%
ESBL-producing *K. pneumoniae strains*	19	15	20	28
All *K. pneumoniae* strains	321	299	359	175

Fig. 2 Annual isolation of ESBL-producing *K. pneumoniae* strains

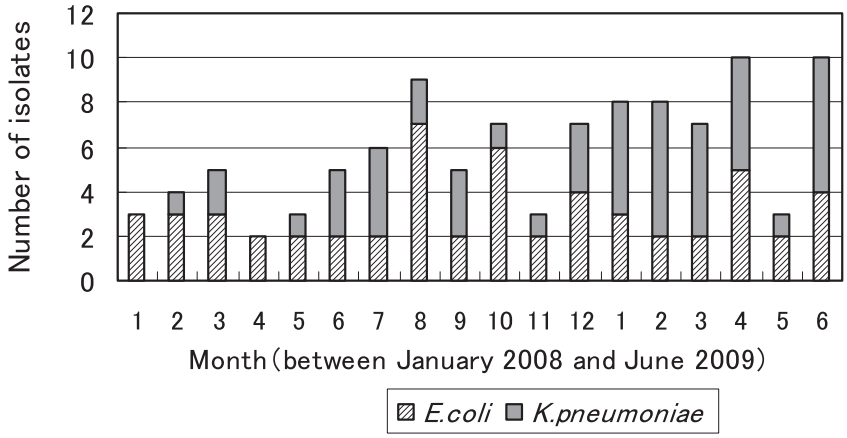


Fig. 3 Monthly ESBL isolation

Table 1 Analysis of ESBL resistance genes

	E. coli (%)		*K. pneumoniae* (%)	
CTX M-1	6 (10.7)	21.4% (*Included)	0	63.3%
CTX M-1 + TEM	5 (8.9)		20 (40.8)	
CTX M-1 + SHV	0		1 (2.0)	
CTX M-1 + TEM + SHV	0		10 (20.4)	
CTX M-2	1 (1.8)	7.1%	0	4.1%
CTX M-2 + TEM	3 (5.4)		0	
CTX M-2 + SHV	0		1 (2.0)	
CTX M-2 + TEM + SHV	0		1 (2.0)	
CTX M-9	14 (25.0)	53.6% (*Included)	2 (4.1)	6.1%
CTX M-9 + TEM	15 (26.8)		0	
CTX M-9 + SHV	0		1 (2.0)	
CTX M-9 + TEM + SHV	0		0	
TEM	9 (16.1)	16.1%	0	26.5%
SHV	0		8 (16.3)	
TEM + SHV	0		5 (10.2)	
*CTX M-1 + M-9 + TEM	1 (1.8)		0	
Unknown	2 (3.6)		0	

Discussion

In our clinic, the prevalence of drug-resistant strains seems to be decreasing because of the restricted use of carbapenem antibiotics. However, the prevalence of ESBL-producing bacteria is increasing. The β-lactamase gene encoded by the R plasmid of ESBL-producing bacteria has acquired the ability to degrade third- and fourth-generation cephem antibiotics because of the mutations. The isolation frequencies of TEM- and SHV-type ESBL-producing bacteria are usually considered to be low in Japan and high in the West. However, in our results, their frequencies were rather high, which might be because the PCR reaction could have detected the prototype β-lactamases without the mutations as well as those with the mutations. This point should be clarified by analyses, such as the sequencing of PCR products.

The amino acid sequence of the CTX M type in Japan (Toho type) is different from that of the ESBL in the West (TEM and SHV types). However, the CTX M type is regarded as an ESBL because it exhibits common properties. The isolation frequencies were also high according to our results: 82.1% for *E. coli* (predominantly CTX M-9 type) and 73.5% for *K. pneumoniae* (predominantly CTX M-1 type). The TEM and SHV genes were coexpressed in most of them. This is presumably because multiple resistance genes, including the original one, were expressed in each bacterial strain.