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Abstracts of Outstanding Presentation (1)

Analysis of Clinically Isolated Extended-spectrum β-lactamase-producing Bacteria

Kenta Sugino[1], Ken Kubomura[1], Tokuhiro Kimura[1],
Yoshihiko Norose[2], Eri Watanabe[2] and Hidemi Takahashi[2]

[1]Nippon Medical School
[2]Department of Microbiology and Immunology, Nippon Medical School

Objectives

Significant efforts are needed to control recently emerged extended-spectrum β-lactamase (ESBL)-producing bacteria, because these organisms are resistant to even 3rd- and 4th-generation cephalosporin derivatives, and the detection rate has been increasing year by year in Japan. In addition, ESBL-encoding genes are located within transposons or integrons, which strongly facilitate the cross-transmission of antibiotic-resistance genes between bacterial species, thereby permitting the spread of resistance among related and unrelated gram-negative bacteria.

In this report, to study the ESBL-producing bacteria more precisely, we attempted to confirm the presence of ESBL genotypes in bacteria and to observe plasmid transfer of ESBL genes.

Materials and Methods

Bacterial Strains

Escherichia coli (n=45) and *Klebsiella pneumoniae* (n=29) producing ESBL were isolated from July 2009 through April 2010 at Nippon Medical School Hospital. An *E. coli* strain (KM^+CTX^-) termed "TOP 10F" was used as a conjugal transfer strain.

Multiplex-polymerase Chain Reaction

We used primer sets for ESBL genotypes (CTX-M-1, CTX-M-2, CTX-M-9, SHV, and TEM). After 30 cycles of the polymerase chain reaction (PCR), the PCR products were resolved with electrophoresis on agarose gels and visualized with ethidium bromide staining under UV light.

Table 1 Prevalence of ESBL genes

	E.coli		*K.pneumoniae*	
	n	%	n	%
CTM-M-1 only	3		0	
CTM-M-1 + SHV	0		1	
CTM-M-1 + TEM	2		11	
CTM-M-1 + SHV + TEM	0		13	
Total of CTM-M-1 group	5	11.1	25	86.3
CTM-M-2 group	0	0	0	0
CTM-M-9 only	13		0	
CTM-M-9 + SHV	0		1	
CTM-M-9 + TEM	17		0	
CTM-M-9 + SHV + TEM	0		1	
Total of CTM-M-9 group	30	66.7	2	6.9
CTM-M-1 + CTM-M-9 + SHV + TEM	0		1	3.4
SHV	0		0	
TEM	4	8.9	0	
SHV + TEM	1	2.2	1	3.4
undefined	5	11.1	0	
Total	45	100	29	100

Antimicrobial Susceptibility Test

The antimicrobial susceptibility of these isolates was analyzed with the disk diffusion method on Mueller-Hinton agar. The antibiotic disks used contained kanamycin (KM), ampicillin (ABPC), clavulanic acid amoxicillin (ACV) (β-lactamase inhibitor), aztreonam (AZT), and 3rd-generation-antimicrobial agents: cefotetanime (CTX), ceftazidime (CAZ), cefpodoxime (CPDX), and ceftriaxone (CTRX). To identify bacteria with ESBLs, ACV was placed in the middle of an agar, and the disks of the third-generation-antibiotics were placed at the periphery. In this way, ESBL-positive bacteria were identified.

Plasmid Transfer Test

For the plasmid transfer test (PTT), conjugal transfer strain of $KM^{+}CTX^{-}$ *E. coli* and clinically-isolated $KM^{-}CTX^{+}$ ESBL-producing *E. coli* (strain number: *Ec*60, *Ec*68, *Ec*70) or *K. pneumoniae* (strain number: *Kp*59, *Kp*74) were cultured together. Then, the strains were cultured together again for 18 hours on Mueller-Hinton agar containing KM and CTX.

Pulse-field Gel Electrophoresis

For pulse-field gel electrophoresis (PFGE), *Spe*I-digested genomic DNA was prepared according to the standard method, and fragments were separated for 20 hour at 6 V/cm.

Results

Patients, Bacterial Isolates, and ESBL Genes

We analyzed ESBL-producing *E. coli* and *K. pneumoniae* isolated at Nippon Medical School Hospital. More than half of the clinical isolates of *E. coli* (61%) were isolated from urine, but only 25% of *K. pneumoniae* were from urine, although they were also isolated from sputum (18%), arterial blood (15%), or venous blood (10%).

Multiplex-PCR (**Table 1**) show that 66.7% of ESBL-producing *E. coli* were positive for the CTX-M-9 group. In

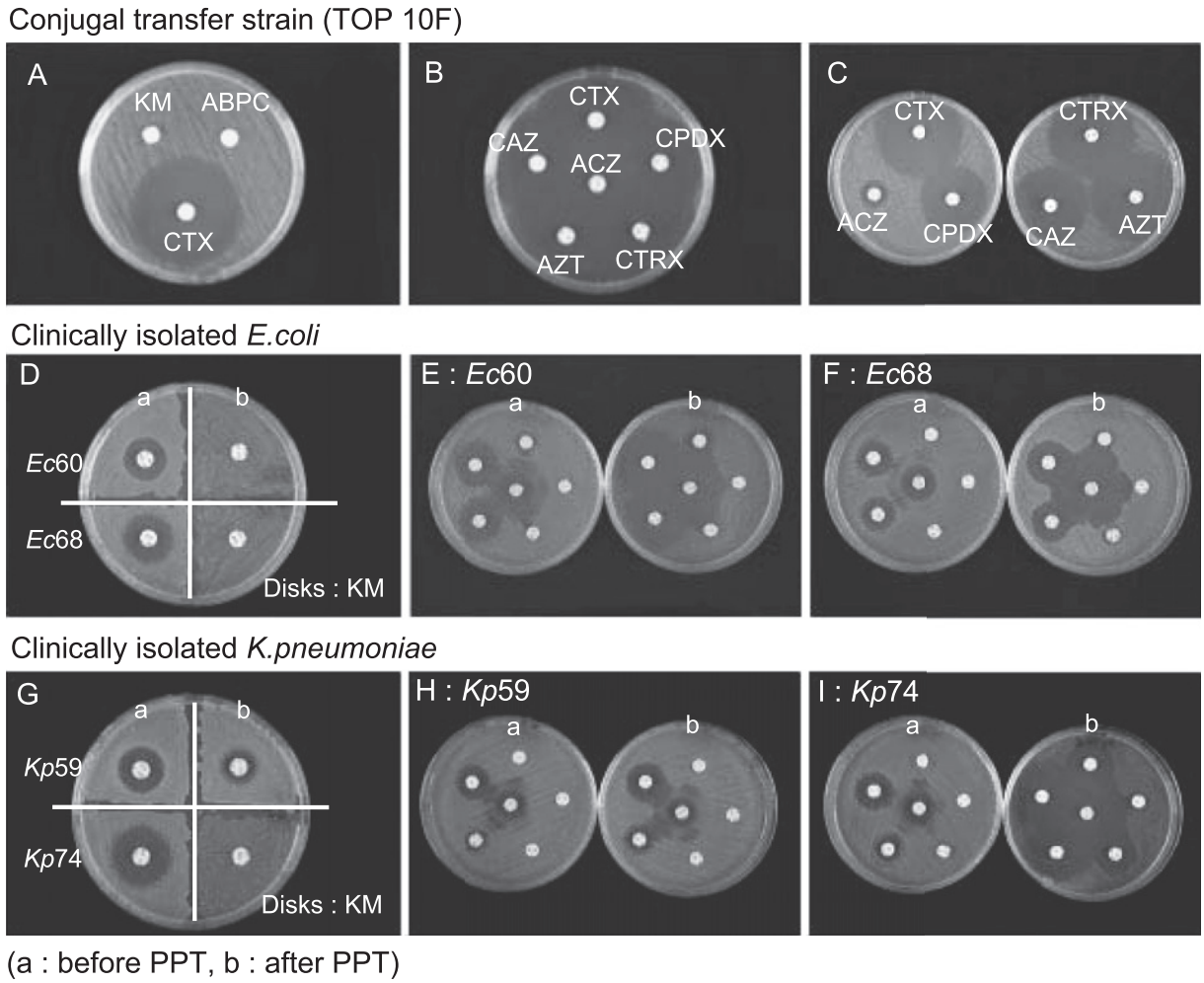

Fig. 1 Antimicrobial susceptibility test

contrast, 86.3% of ESBL-producing *K. pneumoniae* were positive for the CTX-M-1 group.

PTT

A conjugal transfer strain was resistant to KM but susceptible to CTX **(Fig. 1A–C)**. Bacteria resistant to KM and CTX emerged after the co-culture with the conjugal transfer strain (KM^+CTX^- *E. coli*) and the clinically isolated ESBL-producing bacteria (KM^-CTX^+ *E. coli* or *K. pneumoniae*) **(Fig. 1D, G)**. Sensitivity patterns against various antimicrobials before and after PPT are also shown in **Figure 1E, F, H, and I**.

PFGE

The PFGE of the isolates was also performed **(Fig. 2A)**. The DNA pattern of the conjugal transfer strain **(Fig. 2** K+) and those of *Ec*60, *Ec*68, *Ec*70 and *Kp*74 after PPT **(Fig. 2** each b) corresponded with each other. No changes in DNA patterns between the *Kp*59 strains before and after PPT were observed **(Fig. 2A** *Kp*59 a, b). The ESBL genotype was also seen with multiplex-PCR to clarify the transfer of the plasmid **(Fig. 2B)**.

Discussions

Although SHV- and TEM-type were detected, CTX-M-9 and CTX-M-1 were dominant among the ESBL genotypes.

The results indicate that the ESBL plasmid was transmitted to a conjugal transfer strain; however, the DNA

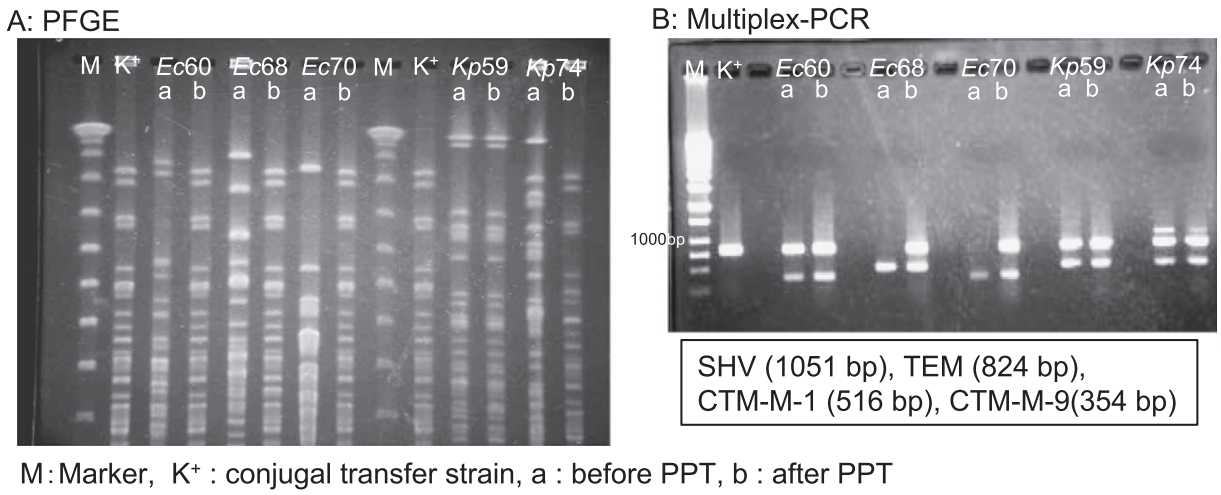

Fig. 2 DNA patterns and ESBL genes before and after PPT

pattern of the Kp59 strain did not change, a finding that indicates that KM-resistant plasmid of the conjugal transfer strain was transmitted to the Kp59 strain. We have shown that resistance genes are easily transmitted via the plasmid exceeding not only the same kind but also the different kind of bacteria. Thus, it is possible that resistance genes could spread to multiple bacterial strains in the infected host with ESBL-producing bacteria, which would make the treatment of the host more difficult.

Therefore, it is important to prevent the spread of bacteria through strict infection management following standard precautions and the appropriate use of antibiotics.