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Major article

Emergence of extended-spectrum β-lactamase-producing *Escherichia coli* in catheter-associated urinary tract infection in neurogenic bladder patients

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Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*
Repetitive-sequence-based polymerase chain reaction (rep-PCR)

Background: Catheter-associated urinary tract infection (CAUTI) is a common clinic problem. The purpose of this study was to investigate recent trends in CAUTI in neurogenic bladder patients focusing on extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*.
Methods: Isolates from the urine of neurogenic bladder patients with UTI were investigated. Nine strains of ESBL-producing *E coli* were assayed by molecular strain typing using the Diversilab system for repetitive-sequence-based polymerase chain reaction (rep-PCR).
Results: *E coli* accounted for most of the bacteria (74.1% to 81.0%) that produced ESBLs. Rep-PCR data showed that 7 out of 9 ESBL-producing *E coli* belonged to the same typing group with high similarity (more than 97% similarity) and that this distribution corresponded with antibiotic resistance patterns.
Conclusion: ESBL producing *E coli* strains isolated from CAUTI patients could be discriminated by rep-PCR typing using the Diversilab system in consistent with antibiotic resistance patterns.

Studies of increasing prevalence of resistant strains in urinary tract infection (UTI) have noted that catheter-associated UTI (CAUTI) has different trends in causative bacteria from non-CAUTI.[1,2] In both, *Escherichia coli* is a representative causative bacteria and sometimes represent resistance to antibiotics such as extended-spectrum β-lactamase (ESBL)-producing *E coli*.[3,4] Such resistant strains need to be monitored closely in hospitalized infections to prevent their spread,[5,6] and epidemiologic analyses are critical for detecting and preventing the emergence of this kind of strain.

Repetitive-sequence-based polymerase chain reaction (rep-PCR) using the Diversilab system (bioMérieux, Marcy l'Etoile, France) is rapid, noncomplex, and cost-effective. It has the advantage of being less labor intensive than pulse-field gel electrophoresis.[7-10]

In this study, we investigated recent causative bacteria including ESBL-producing *E coli* in CAUTI of neurogenic bladder patients and applied rep-PCR the Diversilab system for epidemiologic analyses.

METHODS

Isolates

From 2006 to 2009, a total of 1,108 urine samples was cultured for the diagnosis of suspicious CAUTI patients who presented UTI symptoms such as fever or lower abdominal or back pain with neurogenic bladder in Hyogo Prefectural Rehabilitation Hospital. The isolated bacteria were taken from 10^5 or more colony-forming units/mL in urines.

Susceptibilities to antibiotics

Susceptibility testing was performed for 12 kinds of antibiotics such as sulbactam/ampicillin and cefozopran according to Clinical and Laboratory Standards Institute criteria.[11]

ESBL detection

ESBL-producing bacteria were diagnosed with a positive ESBL screen test. Results were considered positive with formation of inhibition zone by clavulanic acid on the middle amoxicillin/clavulanic acid disc surrounded by the discs of cefotaxime, cefotaxime/clavulanic

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Conflicts of interest: None to report.

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Table 1
Isolated ESBL-producing bacteria from CAUTI

Bacteria	2006, n (%)	2007, n (%)	2008, n (%)	2009, n (%)	r	*P* value	b
Escherichia coli	7 (77.7)	20 (74.1)	15 (78.9)	17 (81.0)	0.656	.344	0.293
Klebsiella pneumoniae	2 (22.2)	4 (14.8)	2 (10.5)	4 (19.0)	0.353	.647	−0.090
Klebsiella oxytoca	0	2 (7.41)	1 (5.26)	0	0.072	.928	−0.025
Proteus mirabilis	0	1 (3.70)	1 (5.26)	0	0.077	.923	0.037
Total	9	27	19	21			

b, regression coefficient; *ESBL*, extended-spectrum β-lactamase; *r*, correlation coefficient.

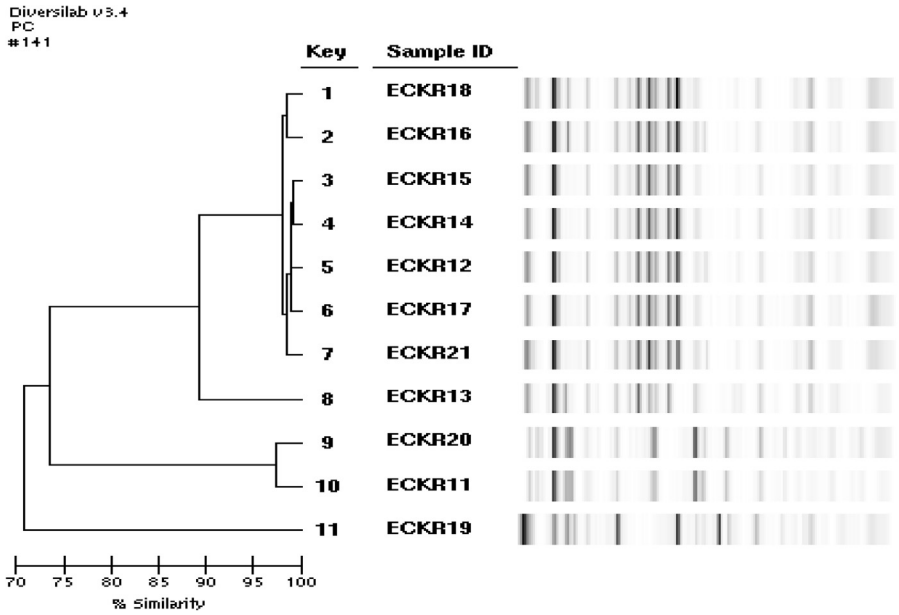

Fig 1. Strain differentiation of ESBL-producing *E coli* isolate using the Diversilab system of auto matched repetitive-sequence-based PCR. ECKR 11-19 showed different samples from different patients; and ECKR 20 was the same as ECKR 11, and ECKR 21 was same as ECKR 12. These different 9 (ECKR 11-19) samples were classified to 3 clusters with 97% similarities.

acid, ceftazidime, cefpodoxime, aztreonum, and piperacillin based on the double disc synergy test.[12]

Rep-PCR

The ESBL strains were subcultured in 5% sheep blood agar medium with 48 hours of incubation. The agar plates were processed for genomic DNA extraction and preparation using the UltraClean microbial DNA isolation kit (bioMérieux). Rep-PCR amplification was performed according to the manufacturer's instructions (bioMérieux). The amplified rep-PCR products analyzed by DiversiLab analysis software (version 3.4; bioMérieux) for a genealogic tree formation.

Statistical analysis

Statistical analyses were conducted by Student *t* and χ^2 test with JSTAT-Java Virtual Machine Statistics Monitoring Tool (Sun Microsystems, Inc, Santa Clara, CA) and linear regression analysis especially for distribution of ESBL-producing bacteria in 4 years with the PASW Statistics 17.0 software package (for Windows; SPSS Inc, Chicago, IL). Statistical significance was established at the .05 level.

RESULTS

Bacterial isolates

In the 4-year study period, 1,524 causative bacteria specimens from the urines of CAUTI patients were isolated. In detail, *E coli* was the most often isolated followed by *Pseudonomas aeruginosa*, *Enterococcus faecalis*, *Klebisiella pneumoniae*, or methicillin-resistant *Staphylococcus aureus*. The ratio of *E coli* isolation was 27.4%, 30.0%, 31.0%, and 35.3% in 2006, 2007, 2008, and 2009, respectively (data not shown).

ESBL distribution

ESBL-producing bacteria were isolated in 9, 27, 19, and 21 cases in 2006, 2007, 2008, and 2009, respectively. Especially, ESBL-producing *E coli* represented most frequently isolated, followed by *K pneumoniae*, and this trend was not changed in 4 years (Table 1). The ESBL-producing *E coli* isolated ratio in all the *E coli* isolated tended to be greater (20.0% to 31.0%) in nosocomial cases than community-acquired cases (13.0% to 25.0%) from 2006 to 2010, but this difference was not statistically significant ($P > .05$).

Rep-PCR

The results showed that our 9 strains were discriminated into 3 groups based on their similarities and that 7 strains were categorized as the same typing with high similarity (more than 97%). Importantly, this rep-PCR discrimination corresponded with antibiotic resistance patterns (Fig 1).

DISCUSSION

Neurogenic bladder patients often have dysfunctional voiding or storage[13] and residual urines, resulting in the presence of pyuria or bacteriuria. Many of these patients need to be catheterized for