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Surveillance

Second nationwide surveillance of bacterial pathogens in patients with acute uncomplicated cystitis conducted by Japanese Surveillance Committee from 2015 to 2016: antimicrobial susceptibility of *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus saprophyticus*☆

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ABSTRACT

The Japanese Surveillance Committee conducted a second nationwide surveillance of antimicrobial susceptibility patterns of uropathogens responsible for acute uncomplicated cystitis (AUC) in premenopausal patients aged 16–40 years old at 31 hospitals throughout Japan from March 2015 to February 2016. In this study, the susceptibility of causative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*) for various antimicrobial agents was investigated by isolation and culturing of organisms obtained from urine samples. In total, 324 strains were isolated from 361 patients, including *E. coli* (n = 220, 67.9%), *S. saprophyticus* (n = 36, 11.1%), and *K. pneumoniae* (n = 7, 2.2%). The minimum inhibitory concentrations (MICs) of 20 antibacterial agents for these strains were determined according to the Clinical and Laboratory Standards Institute (CLSI) manual. At least 93% of the *E. coli* isolates showed susceptibility to fluoroquinolones and cephalosporins, whereas 100% of the *S. saprophyticus* isolates showed susceptibility to fluoroquinolones and aminoglycosides. The proportions of fluoroquinolone-resistant and extended-spectrum β-lactamase (ESBL)-producing *E. coli* strains were 6.4% (13/220) and 4.1% (9/220), respectively. The antimicrobial susceptibility of *K. pneumoniae* was retained during the surveillance period, while no multidrug-resistant strains were identified.

In summary, antimicrobial susceptibility results of our second nationwide surveillance did not differ significantly from those of the first surveillance. Especially the numbers of fluoroquinolone-resistant and ESBL-producing *E. coli* strains were not increased in premenopausal patients with AUC in Japan.

1. Introduction

An uncomplicated urinary tract infection (UTI) is one of the most common diseases caused by bacteria encountered in outpatient settings. Acute uncomplicated cystitis (AUC) accounts for the greatest number of uncomplicated UTI cases, particularly among young sexually active individuals [1]. The condition is diagnosed and treated by physicians of various types, including urologists and gynecologists, as well as other medical and health care providers [2]. Although AUC should be treated with an effective antimicrobial agent, management of AUC patients has recently become more difficult, because of increasing bacterial resistance to antimicrobial agents in case of uncomplicated UTI [34], increasing the importance of more appropriate and effective antimicrobial agents to control AUC.

Several studies throughout the world have reported increased isolation of fluoroquinolone-resistant or extended-spectrum β-lactamase (ESBL)-producing gram-negative bacilli in patients with AUC [5–8]. Therefore, awareness of a specific regional resistance ratio is important prior to initiation of empiric antimicrobial therapy for treatment of AUC. In order to comprehensively investigate antimicrobial susceptibility and resistance of bacterial urinary pathogens, the Japanese Society of Chemotherapy (JSC), Japanese Association for Infectious Diseases (JAID) and Japanese Society for Clinical Microbiology (JSCM) established the Japanese Surveillance Committee in 2008. The first survey conducted in Japan of the antimicrobial susceptibility patterns of the uropathogens *Escherichia coli* and *Staphylococcus saprophyticus*, responsible for female AUC, was conducted during the period from April 2009 to November 2010 [4]. Information regarding the antimicrobial susceptibility of pathogens isolated from AUC patients is of particular value, as that is reflected in guidelines presented for treatment. The second nationwide surveillance of AUC pathogens was conducted during the period from March 2015 to February 2016, with *Klebsiella pneumoniae* added to *E. coli* and *S. saprophyticus* for surveillance of multidrug-resistant pathogens over time.

2. Materials and methods

2.1. Japanese Surveillance Committee

The Japanese Surveillance Committee, consisting of the JSC, JAID and JSCM, conducted a second nationwide surveillance of antimicrobial susceptibility patterns of uropathogens responsible for female AUC. The study protocol was prepared by the working group and accepted by the governing board of the committee. Ethics approval was obtained from the ethical committee of each facility or from the ethical committee of a specific non-profit organization, CREC net, located in Kitakyushu, Japan. The clinicians who participated in this study explained its purpose to their patients orally or through written documents, and obtained written consent for participation. All results were included in the study database, with patient information remaining anonymous.

2.2. Patients

Among the patients with AUC who visited any of 31 related institutions across Japan (Table 1) from March 2015 to February 2016, those with symptoms such as micturition pain, frequent urination, urgency, or suprapubic pain, and who met the following inclusion criteria were enrolled.

The inclusion criteria were set in accordance with the Japanese guidelines for clinical research of antimicrobial agents developed for urogenital infections, first edition [9]. Patients with AUC were required to be female, aged 16–40 years old, without underlying urinary tract disease and/or factors contributing to onset, worsening, or prolongation of urinary tract infection, and without diabetes mellitus, malignancy receiving treatment, cerebrovascular disease requiring assistance, or presently undergoing corticosteroid or immunosuppressant therapy. One or more of the following was also required: pyuria confirmed by a reagent strip; ≥5 white blood cells (WBCs) per high-power field (hpf) in a microscopy examination of urine sediment, or ≥10 WBCs/mm^3 measured by flow cytometry or a counting chamber; and bacteriuria shown by ≥ 10^3 colony-forming units (cfu)/ml (for midstream urine, ≥10^4 cfu/ml).

Table 1
Participating Japanese medical institutions.

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Tsujinaka Hospital Kashiwanoha, Chiba
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Ito Urology Clinic, Fukuoka
Kawai Urology Clinic, Fukuoka
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Listed in alphabetical order, location noted by prefecture.

2.3. Bacteriological examination

2.3.1. Collection of urine samples and bacterial strains

Urine samples were collected from patients with AUC as midstream urine or via a catheter in accordance with the Japanese guideline for clinical research of antimicrobial agents on urogenital infections, first edition [9]. They were examined using a Slide Culture U® system (Eiken Chemical Co. Ltd., Tokyo, Japan) by the investigators and then sent on the same day to a central bacteriological laboratory (Infection Control Research Center, Kitasato Institute for Life Science, Kitasato University, Tokyo, Japan) for testing. If the same patient participated a second time in the study, a duplicate specimen was not used.

Bacterial strains thus obtained were cultured at 35 ± 2 °C for 16–24 h, then isolates were identified, counted, and stored at −80 °C for future examination.

2.3.2. Susceptibility testing and determination of minimal inhibitory concentration (MIC)

The MICs of antimicrobial agents used against the 3 main causative organisms of AUC examined, *E. coli*, *K. pneumoniae,* and *S. saprophyticus*, were determined using a broth microdilution method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) set out in standards M7-A7 [10], M100-S17 [11], and M45-A [12], respectively, using cation-adjusted Mueller-Hinton broth (25 mg/l Ca^{++} and 12.5 mg/l Mg^{++}; CA-MH broth). Bacterial cells were grown overnight, adjusted to approximately 10^4 cfu/well (10^5 cfu/ml) and incubated at 35°±2 °C for 16–20 h.

2.3.3. Quality control

The accuracy of determination of the MICs of the antimicrobial agents was determined according to the recommendations of the CLSI, using the following control strains: *E. coli* ATCC25922 for *E. coli* and *K. pneumoniae*, and *S. aureus* ATCC29213 for *S. saprophyticus*. Furthermore, *E. coli* ATCC35218 was utilized as a control strain for MIC determination of β-lactam antibiotics used in combination with β-lactamase inhibitors.

2.3.4. Antibacterial agents

Susceptibility of the *E. coli* strains for the following 17 antimicrobial agents were tested: ampicillin (ABPC); 1 penicillin in combination with β-lactamase inhibitors, clavulanic acid-amoxicillin (CVA/AMPC); 5 oral cephalosporins, cefaclor (CCL), cefpodoxime (CPDX), cefdinir (CFDN), cefditoren (CDTR), and cefcapene (CFPN); 1 penem, faropenem (FRPM); 2 aminoglycosides, gentamicin (GM), and amikacin (AMK); 4 fluoroquinolones, ciprofloxacin (CPFX), levofloxacin (LVFX), tosufloxacin (TFLX), and sitafloxacin (STFX); and 3 others, fosfomycin (FOM), sulfamethoxazole/trimethoprim (ST) and nitrofurantoin (NIT).

Additionally, the susceptibilities of *K. pneumoniae* strains was tested with 16 antimicrobial agents in the same manner as the *E. coli* testing, except for NIT.

The susceptibility of *S. saprophyticus* strains was tested with 19 antimicrobial agents in the same manner as with *E. coli* testing, except for NIT, with oxacillin (MPIPC); and 2 glycopeptides, vancomycin (VCM) and teicoplanin (TEIC), added.

2.3.5. Detection of β-lactamases production

A Cica-Beta Test (Kanto Chemical, Tokyo, Japan), a rapid method for detection of extended-spectrum β-lactamase (ESBL) -producing *E. coli* and *K. pneumoniae*, was used by directly scraping a colony and applying it to the disk [13,14]. A modified Hodge Test, a method for detection of carbapenemase-producing Gram-negative bacilli, was used for detection of *Klebsiella pneumoniae* carbapenemase (KPC) -producing *K. pneumoniae*. These tests were conducted according to the reference manual supplied by the manufacturer.

2.4. Patient characteristics

On the day of urine collection, each patient was interviewed by an investigator and the following characteristics were noted: age, menopausal status, and method used for urine sample collection. Patient clinical findings and identified bacterial species were recorded with a standardized data sheet. Microbiological data obtained were analyzed according to the patient clinical settings and profile.

2.5. Assessment

2.5.1. Assessment of antimicrobial susceptibility

The antimicrobial susceptibility of the pathogens was categorized into 1 of 3 classes; namely, susceptible, intermediate, or resistant, according to the MIC breakpoints recommended by CLSI standards M100-S26 [15], and susceptibility rate was defined as the percentage of susceptible strains. For agents not described in the CLSI guidelines, breakpoints were set based on those for similar agents. The breakpoints for LVFX in the CLSI guidelines are ≤2 μg/ml (susceptible), 4 μg/ml (intermediate), and ≥8 μg/ml (resistant), and those for CPFX are ≤1, 2, and ≥4 μg/ml, respectively. Thus, the corresponding values for TFLX and STFX were likewise set at ≤1 μg/ml (susceptible), 2 μg/ml (intermediate), and ≥4 μg/ml (resistant). The breakpoints for CPDX in the CLSI guidelines are ≤2 μg/ml (susceptible), 4 μg/ml (intermediate), and ≥8 μg/ml (resistant), and those for CFDN are ≤1, 2, and ≥4 μg/ml, respectively. Thus, the corresponding values for CDTR, CFPN, and FRPM were set at ≤1 μg/ml (susceptible), 2 μg/ml (intermediate), and ≥4 μg/ml (resistant). Fluoroquinolone resistance was defined as that equivalent to the MIC of LVFX for *E. coli* (≥4 μg/ml).

2.5.2. Assessment of antimicrobial resistant rate of E. coli by isolated age

The antimicrobial resistant rate of *E. coli* was statistically compared between the results of the first nationwide survey [3] and those of the present surveillance using a chi-square test for independence. A *P*-value of <0.05 was considered to indicate statistical significance. For that comparison, data obtained in the first nationwide survey were sub-analyzed after dividing the patients into 2 groups, 16 to 40 and over 40 years old.

3. Results

3.1. Study population and patient characteristics

A total of 403 urine obtained from 31 hospitals in Japan during the study period were registered, among which 361 were included in the analysis. Forty-two samples obtained from thirty postmenopausal patients, eleven patients above 40 years old, and one male patient, were excluded. Patient background information is shown in Table 2. All patients with AUC were female and premenopausal, and the mean age (±standard deviation) was 28.9 ± 6.4 years. Nearly all of the urine samples (99.0%) were collected as midstream urine.

3.2. Causative bacteria

Causative bacteria in the patient cohort are shown in Table 3. The number of strains isolated as causative from 361 patients with AUC was 324. Major causative bacteria included 220 *E. coli* (67.9%), 36 *S. saprophyticus* (11.1%), 7 *K. pneumoniae* (2.2%), 16 *Streptococcus agalactiae* (4.9%), 11 *Staphylococcus epidermidis* (3.4%), and 10 *Enterococcus faecalis* (3.1%) organisms. Gram-negative and -positive bacteria accounted for 72.8% (236 strains) and 27.2% (88 strains), respectively, of all identified strains, for a ratio of 7:3.

3.2.1. Susceptibility profile

MIC distribution, i.e. MICs for 50% and 90% of the organisms (MIC_{50} and MIC_{90} values, respectively), is shown in Tables 4–7. The susceptibility rate based on the MIC breakpoints set by CLSI for each antimicrobial agent were stratified by *E. coli*, ESBL-producing *E. coli*, *K. pneumoniae,* and *S. saprophyticus*, and are shown in Figs. 1–4, respectively.

3.2.2. Antimicrobial susceptibility of Escherichia coli (220 strains)

The susceptibility rates of 220 *E. coli* strains isolated from the premenopausal female patients with AUC for oral cephalosporins were high (92.3% for CCL, 92.3% for CPDX, 92.7% for CFDN, 92.7% for CDTR, 94.5% for CFPN), with CFPN showing the highest rate (Fig. 1). The MIC_{90} of a penicillin derivative (ABPC) for these *E. coli* strains was ≥256 μg/ml, whereas the MIC_{90} of a penicillin derivative in the presence ofβ-lactamase inhibitors (CVA/AMPC) was decreased to 16 μg/ml (Table 4). The MIC_{90} of 5 oral cephalosporins and 1 oral penem (FRPM) ranged from 0.5 to 4 μg/ml, thus each was found to be active against *E. coli* (Table 4). The susceptibility rates for all fluoroquinolones were also high (93.6% for LVFX, 99.5% for STFX, 93.6% for CPFX, 93.6% for TFLX), with STFX showing the highest rate (Fig. 1). The MIC_{90} of 5 fluoroquinolones for these *E. coli* strains ranged from 1 to ≥32 μg/mL and the percentage of resistant strains was 6.4%. Susceptibility to 2 aminoglycosides, GM and AMK, was very high with rates of 95.0% and 100%, respectively, while FOM and NIT also showed very high rates of 100% and 99.5%, respectively (Fig. 1).

Nine ESBL-producing strains were detected (9/220; 4.1%). For those strains, the MIC_{90} of ABPC and 5 oral cephalosporins ranged

Table 2
Patient background information.

	Total number of urine samples		Number of analyzed urine samples	
	N	(%)	N	(%)
Gender				
female	402	99.8	361	100
male	1	0.2		
Age, years				
16-19	21	5.2	21	5.8
20-29	164	40.7	164	45.4
30-39	164	40.7	164	45.4
40-49	23	5.7	12	3.3
50-59	8	2.0	–	–
60-	23	5.7		
Collection method				
catheter	4	1.0	3	0.8
midstream urine	399	99.0	358	99.2
Menopausal status				
premenopausal	372	92.3	361	100
postmenopausal	30	7.4	–	–
male	1	0.2	–	–

Table 3
Causative organisms isolated from patients with acute uncomplicated cystitis (AUC).

Organisms	N	(%)
Gram-negative bacteria	236	72.8
Escherichia coli	220	67.9
Klebsiella pneumoniae	7	2.2
Citrobacter koseri	4	1.2
Proteus mirabilis	4	1.2
Enterobacter aerogenes	1	0.3
Gram-positive bacteria	88	27.2
Staphylococcus saprophyticus	36	11.1
Streptococcus agalactiae	16	4.9
Staphylococcus epidermidis	11	3.4
Enterococcus faecalis	10	3.1
Staphylococcus aureus	6	1.9
Staphylococcus sp.	4	1.2
Streptococcus anginosus	3	0.9
Staphylococcus caprae	1	0.3
Enterococcus faecium	1	0.3

from 32 to ≥256 μg/ml (Table 5), and the susceptibility rate for all antimicrobials was decreased, except for FRPM, AMK, STFX, FOM, and NIT. Remarkably, 100% of the examined strains were resistant to ABPC, CCL, CPDX, and CDTR (Fig. 2).

3.2.3. Antimicrobial susceptibility of Klebsiella pneumoniae (7 strains)

The susceptibility rate of 7 *K. pneumoniae* strains isolated from the premenopausal female patients with AUC for the penicillin derivative with the β-lactamase inhibitors, 5 oral cephalosporins, and the oral penem was high (85.7% for CVA/AMPC, CCL, CPDX, CFDN, CDTR, CFPN, FRPM) (Fig. 3). Therefore, all were considered active against *K. pneumoniae* (Table 6). The susceptibility rate for all fluoroquinolones was also high (100% for LVFX, STFX, CPFX, TFLX) (Fig. 3). The MIC_{90} for the fluoroquinolones with these *K. pneumoniae* strains ranged from 0.25 to ≥1 μg/mL and there no resistant strains (0%) were identified. Susceptibility to 2 aminoglycosides (GM, AMK) and FOM was very high at 100%. FOM was also very high with susceptibility rates of 100% (Fig. 3). No ESBL- or KPC-producing strain (0/7; 0.0%) was detected.

3.2.4. Antimicrobial susceptibility of Staphylococcus saprophyticus (36 strains)

The 36 *S. saprophyticus* strains isolated from the premenopausal female patients with AUC showed variable susceptibility to oral β-lactam antibiotics. The MIC_{90} of 3 penicillin derivatives (MPIPC, ABPC, CVA/AMPC) ranged from 0.5 μg/ml, and that of 5 oral

Table 4
Distribution of minimal inhibitory concentrations (MICs) for *Escherichia coli* (n = 220).

Antibacterial agent	M I C (μg/mL)															Breakpoint MIC (μg/mL)		
	≦0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≧256	MIC_{50}	MIC_{90}	Susceptible	Intermediate	Resistant
Ampicillin					2	63	89	5			3	2	56	4	≧256	≦8	16	≧32
Clavulanic acid-amoxicillin					7	53	98	23	28	3	5	3*		4	16	≦8	16	≧32
Cefaclor				40	107	39	16	1		1	4	2	10	1	4	≦8	16	≧32
Cefpodoxime		1	114	83	4	1	2	3	2	2	3	4	1	0.25	0.5	≦2	4	≧8
Cefdinir	5	123	61	14	1	1	2	2	1	1	3	6*		0.125	0.5	≦1	2	≧4
Cefditoren[a]	3	104	92	4	1	4	3	1				8*		0.25	0.25			
Cefcapene[a]		18	120	61	8	3	2	1	4	3				0.25	0.5			
Faropenem[a]			45	156	15	2	1	1						0.5	0.5			
Gentamicin			6	175	26	2			3	1	2	4	1	0.5	1	≦4	8	≧16
Amikacin					38	143	36	3						2	4	≦16	32	≧64
Ciprofloxacin	172	2	25	4	3	1		4	2	4	3			≦0.06	0.25	≦1	2	≧4
Levofloxacin	167	4	7	23	5		5	2	7					≦0.06	0.5	≦2	4	≧8
Tosufloxacin[a]	173	18	10	4	1	1			1	12**				≦0.06	0.25			
Sitafloxacin[a]	197	9		6	7	1								≦0.06	0.125			
Fosfomycin		3	53	86	50	14	3	1	3	5	2			0.5	2	≦64	128	≧256
Sulfamethoxazole/trimethoprim	147	33	7	9	1				23***					0.06	≧16	≦2		≧4

* ≧128, ** ≧32, *** ≧16.
[a] Agents not described in the CLSI guidelines.

Table 5
Distribution of minimal inhibitory concentrations (MICs) for ESBL-producing *Escherichia coli* (n = 9).

Antibacterial agent	M I C (μg/mL)															Breakpoint MIC (μg/mL)		
	≦0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≧256	MIC_{50}	MIC_{90}	Susceptible	Intermediate	Resistant
Ampicillin													9	≧256	≧256	≦8	16	≧32
Clavulanic acid-amoxicillin								4	4	1				16	32	≦8	16	≧32
Cefaclor										1			8	≧256	≧256	≦8	16	≧32
Cefpodoxime								1		1	2	4	1	128	≧256	≦2	4	≧8
Cefdinir				1							3	5*		128	128	≦1	2	≧4
Cefditoren[a]							1					8*		128	128			
Cefcapene[a]				1				1	4	3				16	32			
Faropenem[a]				5	4									0.5	1			
Gentamicin				4					1		1	2	1	16	≧256	≦4	8	≧16
Amikacin					1	6	2							2	4	≦16	32	≧64
Ciprofloxacin	3		2	1						2	1			0.25	64	≦1	2	≧4
Levofloxacin	2	1		2	1				3					0.5	16	≦2	4	≧8
Tosufloxacin[a]	3	1	1		1					3**				0.25	32			
Sitafloxacin[a]	5	1		1	2									≦0.06	1			
Fosfomycin				4	3				2					1	16	≦64	128	≧256
Sulfamethoxazole/trimethoprim	3	2		1					3***					0.125	≧16	≦2		≧4

* ≧128, ** ≧32, *** ≧16.
[a] Agents not described in the CLSI guidelines.

Table 6
Distribution of minimal inhibitory concentrations (MICs) for *Klebsiella pneumoniae* (n = 7).

Antibacterial agent	M I C (μg/mL)															Breakpoint MIC (μg/mL)		
	≦0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≧256	MIC_{50}	MIC_{90}	Susceptible	Intermediate	Resistant
Ampicillin										4	2		1	32	≧256	≦8	16	≧32
Clavulanic acid-amoxicillin						6						1*		2	≧128	≦8	16	≧32
Cefaclor				5	1								1	0.5	≧256	≦8	16	≧32
Cefpodoxime		6							1					0.125	16	≦2	4	≧8
Cefdinir	4	1	1							1				≦0.06	32	≦1	2	≧4
Cefditoren[a]		3	3			1								0.25	2			
Cefcapene[a]			3	3			1							0.5	4			
Faropenem[a]			2	4			1							0.5	4			
Gentamicin			5	2										0.25	0.5	≦4	8	≧16
Amikacin					7									1	1	≦16	32	≧64
Ciprofloxacin	6			1										≦0.06	0.5	≦1	2	≧4
Levofloxacin	4	2			1									≦0.06	1	≦2	4	≧8
Tosufloxacin[a]	6			1										≦0.06	0.5			
Sitafloxacin[a]	6		1											≦0.06	0.5			
Fosfomycin							2	4	1					8	16	≦64	128	≧256
Sulfamethoxazole/trimethoprim		2	4			1								0.25	2	≦2		≧4

* ≧128.
[a] Agents not described in the CLSI guidelines.

Table 7
Distribution of minimal inhibitory concentrations (MICs) for *Staphylococcus saprophyticus* (n = 36).

Antibacterial agent	M I C (μg/mL)															Breakpoint MIC (μg/mL)		
	≦0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≧256	MIC_{50}	MIC_{90}	Susceptible	Intermediate	Resistant
Oxacillin			1	30	3						1		1	0.5	1	≦0.25		≧0.5
Ampicillin[a]		2	29	3			1	1						0.25	0.5			
Clavulanic acid-amoxicillin[1)]			23	11			1	1						0.25	0.5			
Cefaclor[a]				1	24	9			1	1				1	2			
Cefpodoxime[a]					3	21	10				1		1	2	4			
Cefdinir[a]	1	18	15							1		1*		0.125	0.25			
Cefditoren[a]				12	21	1			1			1*		1	1			
Cefcapene[a]			1	16	14	3				1			1	1	2			
Faropenem[a]			27	7		1		1						0.25	0.5			
Gentamicin	33	2			1									≦0.06	≦0.06	≦4	8	≧16
Amikacin		13	22	1										0.25	0.25	≦16	32	≧64
Ciprofloxacin			20	16										0.25	0.5	≦1	2	≧4
Levofloxacin				36										0.5	0.5	≦1	2	≧4
Tosufloxacin[a]	18	18												≦0.06	0.125			
Sitafloxacin[a]	36													≦0.06	≦0.06			
Fosfomycin[a]										5	17	8	6	64	≧256			
Vancomycin					28	7	1							1	2	≦4	8 to 16	≧32
Teicoplanin					10	20	5	1						2	4	≦8	16	≧32
Sulfamethoxazole/trimethoprim	33	3												≦0.06	≦0.06	≦2		≧4

* ≧128.
[a] Agents not described in the CLSI guidelines.

cephalosporins and 1 oral penem (FRPM) from 0.25 to 4 μg/ml. Therefore, all were active against *S. saprophyticus*, except for two cephalosporins (CPDX and CFPN) (Table 7). The MIC_{90} of fluoroquinolones for *S. saprophyticus* ranged from ≤0.06 to 0.5 μg/ml, while fluoroquinolone resistant strains were not seen (Table 7). All examined *S. saprophyticus* strains were susceptible to 2 aminoglycosides, GM and AMK, and 2 glycopeptides, VCM and TEIC (Fig. 4). On the other hand the MIC_{50} and MIC_{90} values for FOM were 64 and ≥ 256 μg/ml, respectively.

3.2.5. Assessment of antimicrobial resistant rate of Escherichia coli by year of isolation

Data obtained in the first surveillance conducted in 2009–2010 were analyzed in terms of age and compared with those from the present surveillance, after dividing the patients into 2 sub-groups aged 16–40 years and over 40 years. In a comparison between the first and second surveillance for patients aged 16–40 years, the antimicrobial resistant rates of *E. coli* to various antimicrobials including fluoroquinolones were not significantly different, except for CVA/AMPC ($P = 0.00388$) (Table 8).

4. Discussion

It is important to understand the antimicrobial susceptibility profiles of causative bacteria for AUC in local areas, because the resistance patterns of such pathogens vary considerably among regions and countries. Accordingly, we investigated trends over time regarding the distribution of causative bacteria and their susceptibility profile in patients with AUC in Japan.

AUC frequently occurs in young sexually active individuals, and is considered to be uncomplicated when sporadic or a community-acquired episode in otherwise healthy individuals with no known structural or functional abnormalities of the genitourinary tract [16,17]. A previous cohort study showed that the rate of incidence of AUC is 0.5–0.7 per individual per year among young women [1]. A diagnosis of AUC can be made with high probability based on a

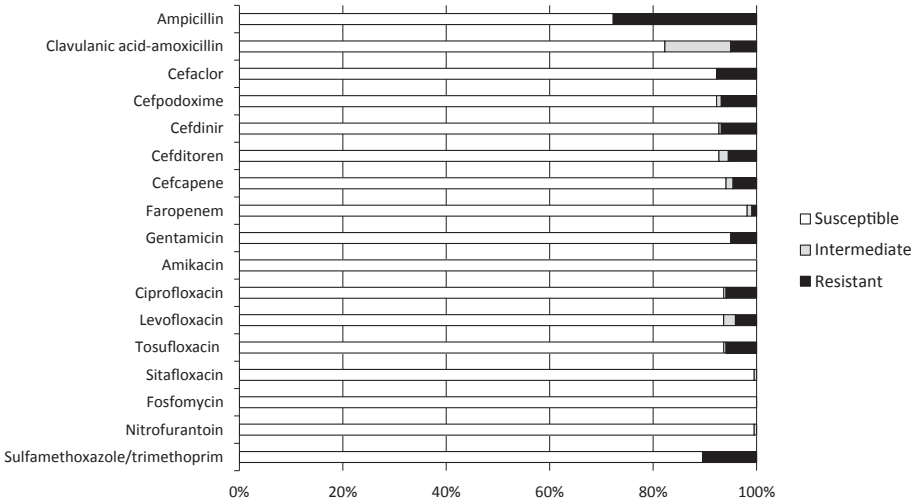

Fig. 1. The antimicrobial susceptibility of *Escherichia coli* was categorized as susceptible, intermediate, or resistant, according to the MIC breakpoints recommended by the CLSI standards M100-S26. Sixteen antimicrobial agents were tested with 220 strains of *E. coli*.

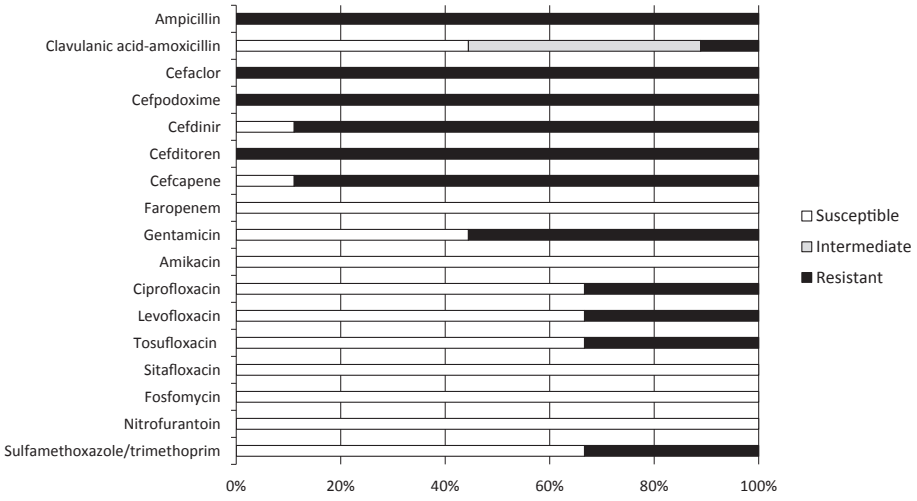

Fig. 2. The antimicrobial susceptibility of ESBL-producing *Escherichia coli* was categorized as susceptible, intermediate, or resistant, according to the MIC breakpoints recommended by the CLSI standards M100-S26. Sixteen antimicrobial agents were tested with 9 strains of ESBL-producing *E. coli*.

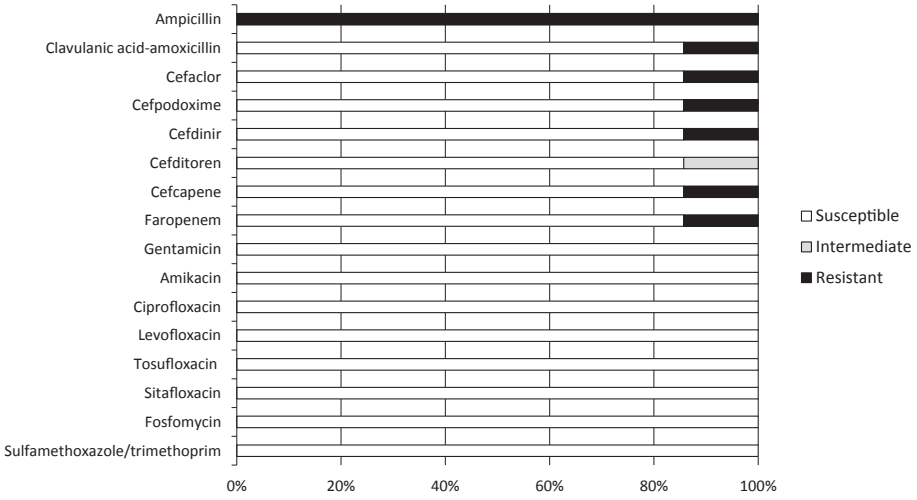

Fig. 3. The antimicrobial susceptibility of *Klebsiella pneumoniae* was categorized as susceptible, intermediate, or resistant, according to the MIC breakpoints recommended by the CLSI standards M100-S26. Sixteen antimicrobial agents were tested with 7 strains of *K. pneumoniae*.

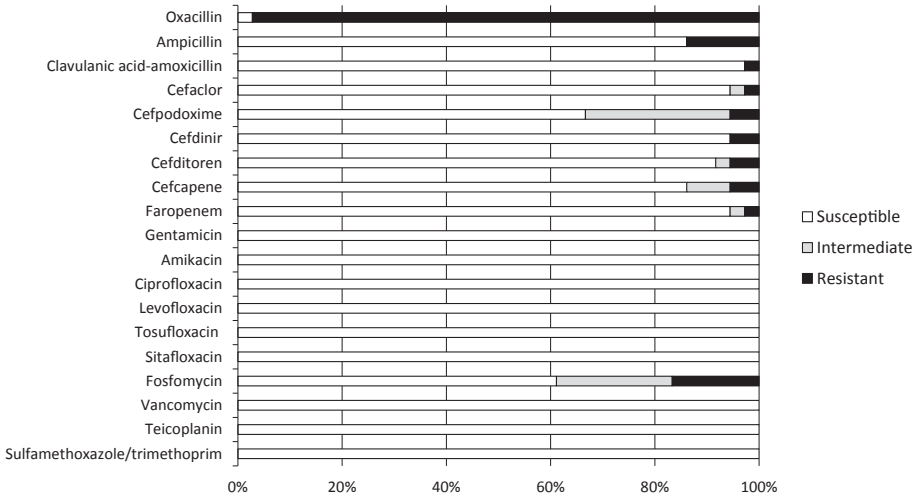

Fig. 4. The antimicrobial susceptibility of *Staphylococcus saprophyticus* was categorized as susceptible, intermediate, or resistant, according to the MIC breakpoints recommended by the CLSI standards M100-S26. Nineteen antimicrobial agents were tested with 36 strains of *S. saprophyticus*.

history of urinary symptoms, physical examination findings, and urinalysis and urine culture results in women younger than 40 years old [18]. On the other hand, symptomatic women aged over 40 years may potentially have an underlying disease of the urinary tract. Since the symptoms of AUC can be mimicked by those of interstitial cystitis, urethral syndrome, and bladder carcinoma, symptomatic patients older than 40 years should be evaluated for a noninfectious cause related to their condition [19]. Postmenopausal women presented with lower urinary tract symptoms are less likely to be cured by antibiotics, particularly short courses of therapy, as compared to premenopausal women [20], thus it is important for physicians to differentiate between uncomplicated and complicated UTI, though the definitions are not equivocal. In order to clarity factors related to a diagnosis of AUC, we conducted the present surveillance in premenopausal women aged 16–40 years old.

The microbial etiology of AUC is regarded to be well established and reasonably consistent. *E. coli* has been reported to be the pathogen isolated most frequently during episodes of AUC, followed by *S. saprophyticus* [19,21], while other pathogens, such as *K. pneumoniae, Proteus mirabilis*, and *Enterococcus faecalis*, are occasionally involved [3,6,21]. In the present study, *E. coli* organisms comprised the largest number (220 of 324; 67.9%) of isolates and the distribution of causative bacteria for AUC was similar to that in mega-surveillance studies conducted in other countries [6,22,23]. As compared with the first nationwide surveillance in Japan [4], the isolation rate of *S. saprophyticus* in patients aged 16–40 years old (sub-analysis) was slightly high in the present study (11.1% vs. 9.4%), indicating that AUC caused by *S. saprophyticus* is showing an increasing trend in premenopausal women in Japan.

Fluoroquinolone-resistant *E. coli* has been noted to be a worldwide clinical problem. However, in the present study, the proportion of fluoroquinolone-resistant or intermediate *E. coli* isolated from patients with AUC was 6.4%, slightly lower than the rates noted in our other recent studies (8.2–12.3%) [3,4]. When subanalysis according to age was performed for the antimicrobial susceptibility of *E. coli* in patients aged 16–40 years old who participated in the first nationwide surveillance in Japan, the susceptibility rates for CCL, CPDX, CFPN, CPFX, and LVFX were 93.8%, 94.7%, 96.5%, 92.0%, and 92.0%, respectively. In addition, the present study showed similar results in terms of susceptibility for cephalosporins as well as fluoroquinolones. While susceptibility to STFX was extremely high (99.5%), the susceptibility rate of *E. coli* isolated from AUC cases towards conventional fluoroquinolones was 93.6%, similar to the rates for oral cephalosporins (92.3–94.1%). On the other hand, *E. coli* showed high rates of susceptibility to FRPM and FOM at 98.2% and 100%, respectively. In the present study, 9 ESBL-producing *E. coli* (4.1%) isolates were identified, which is an isolation rate approximately 4 times higher than previous Japanese findings presented in 2003 [24], though that rate did not show an increasing trend for females aged 16–40 years old as compared to the first surveillance conducted from 2009 to 2010 [4]. Among the ESBL-producing *E. coli* isolates, susceptibility to ABPC, CCL, CPDX, CFDN, CDTR, and CFPN was 0%, whereas that to FRPM, AMK, STFX, FOM, and NIT was 100%. Together, these results indicate that the antimicrobial resistant rates of *E. coli* organisms isolated from premenopausal patients with AUC in Japan have not increased in the most recent 5-year period. Nevertheless, the rate of resistance to CVA/AMPC was significantly increased in the present study, suggesting the necessity to continue surveillance, since that is considered to be a clinically effective agent for treating patients infected with ESBL-producing *E. coli* [25].

Table 8
Stratified analysis of antimicrobial resistant rate of *E. coli* by year of isolation.

Antibacterial agent	Resistant rate (%)			p value
	Year	2009–10	2015–16	
	Patient age, years	16–40	16–40	
	Number of patients	113	220	
Ampicillin		23.0	27.7	NS
Clavulanic acid-amoxicillin		6.2	17.7	0.00388
Cefaclor		6.2	7.7	NS
Cefpodoxime		5.3	7.7	NS
Cefdinir		4.4	7.3	NS
Cefditoren		2.7	7.3	NS
Cefcapene		3.5	5.9	NS
Faropenem		1.8	1.8	NS
Gentamicin		3.5	5.0	NS
Amikacin		0.0	0.0	
Ciprofloxacin		8.0	6.4	NS
Levofloxacin		8.0	6.4	NS
Tosufloxacin		8.0	6.4	NS
Sitafloxacin		0.0	0.5	NS
Fosfomycin		0.0	0.0	
Sulfamethoxazole/trimethoprim		9.7	10.5	NS

NS: P value > 0.05.

K. pneumoniae is commonly isolated from UTI cases, as well as patients with respiratory tract, surgical site, bacteremia, and other types of infection. Recently, antimicrobial-resistant *K. pneumoniae* strains have emerged and reported to be spreading throughout the world [26–28]. In the samples obtained in the present study from premenopausal women with AUC, only 7 of the *K. pneumoniae* strains isolated (2.2%) were found to not show multidrug-resistance, while neither ESBL- nor KPC-producing strains were isolated.

Based on the reported detection rate of pathogens by menopausal status, AUC caused by *S. saprophyticus* is significantly more common in premenopausal women [4]. In addition, the *S. saprophyticus* strains isolated in the present investigation were found have a high susceptibility to fluoroquinolones and aminoglycoside antibiotics, as compared to other β-lactams and FOM. On the other hand, both *E. coli* and *S. saprophyticus* showed a rate of resistance to ampicillin (ABPC) of about 30%, thus that drug should no longer be recommended for empiric therapy for AUC as a single agent without a β-lactamase inhibitor [4,6].

According to the Guidelines for Antimicrobial Use published by JAID and JSC in 2015 [29], the standard antimicrobial regimen for treatment of AUC in premenopausal women is 3 days of therapy with fluoroquinolones or 5–7 days with β-lactam antibiotics. When Gram-positive cocci are suspected or have been detected, 3 days of therapy with fluoroquinolones is recommended for both pre- and postmenopausal women. The results of the present second surveillance in Japan suggest the aptness of such empirical treatment. Fluoroquinolones are most effective against Gram-positive cocci in terms of antimicrobial effect. On the other hand, use of fluoroquinolones was associated with collagen-related disorders [30] and an increased risk of aortic aneurysm and dissection [31]. Physicians should be aware of this possible drug safety risk associated with fluoroquinolone therapy. In addition, in order to suppress induction of fluoroquinolone resistance, physicians should refrain from using fluoroquinolones as routines for elderly women at postmenopausal status with AUC caused by Gram-positive cocci and alternatively consider taking AMPC/CVA. Thus, for appropriate selection of an antimicrobial agent, it is important for the treating physician to distinguish causative pathogens of AUC between coccus- and coli-forms. For that, microscopy findings of urine sediment with or without Gram staining is recommended, not only to ensure diagnostic accuracy, but also to distinguish between those forms. In the future, flow cytometry may take the place of microscopy, because a system for discriminating between Gram-positive and negative bacteria has been developed and may be available soon for clinical use [32].

5. Conclusions

AUC is of significant importance in the community due to its high prevalence, particularly among young sexually active women. To appropriately use antimicrobial agents for empiric therapy, it is important to confirm the susceptibility profile of the causative bacterium prior to treatment and also carefully consider patient characteristics. Regarding the premenopausal group, antimicrobial susceptibility results of our second nationwide surveillance did not differ significantly from those of the first surveillance, which was conducted 5 years earlier, though antimicrobial resistant gram-negative bacilli have increased in AUC patients throughout the world. Especially the numbers of fluoroquinolone-resistant and ESBL-producing *E. coli* strains were not increased in Japan. The results presented in this study provide important information for proper treatment of UTIs and can be a useful reference for future surveillance studies.

Conflicts of interest

Satoshi Takahashi has received research funding from Abbott Japan Co., Ltd., and received scholarship donations from Shino-Test Corporation.

Shingo Yamamoto has received speaker's honorarium from Astellas Pharma Inc. and Daiichi Sankyo Co., Ltd., and received scholarship donations from Astellas Pharma Inc., Takeda Pharmaceutical Co., Ltd., Novartis Pharma K.K., Daiichi Sankyo Co., Ltd., Pfizer Japan Inc., and Bayer Yakuhin, Ltd.

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