



Original Article

Investigation of the susceptibility trends in Japan to fluoroquinolones and other antimicrobial agents in a nationwide collection of clinical isolates: A longitudinal analysis from 1994 to 2016



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ABSTRACT

The susceptibilities of clinical isolates to fluoroquinolones and other antimicrobial agents were surveyed to obtain an accurate understanding of trends in incidence and antimicrobial resistance. The samples were collected from across Japan, biennially or triennially, between 1994 and 2016 and a defined level of resistance to fluoroquinolone was determined.

Streptococcus pneumoniae, *Streptococcus pyogenes* and *Haemophilus influenzae* exhibited stable and high rates of susceptibility to fluoroquinolones over the period examined. For methicillin-resistant *Staphylococcus aureus* the rate of resistance to levofloxacin and ciprofloxacin was 81.3–93.5% and 83.2–94.2%, respectively, which was markedly higher than that of methicillin-susceptible *S. aureus*, while sitafloxacin-resistant methicillin-susceptible and methicillin-resistant *S. aureus* were isolated at 0.3–0.7% and 16.9–36.5%, respectively. The rate of levofloxacin or ciprofloxacin-resistant *Escherichia coli* increased from around 2–3% between 1994 and 1998 to around 35% in 2016, but the rate of fluoroquinolone-susceptible *Klebsiella pneumoniae* stayed high at over 94.6% during the study period. Although no fluoroquinolone-resistance in clinical isolates of *Salmonella* spp. was detected from 1994 to 2002, the resistance rate increased slightly after 2004 and reached to 1.9–4.7% in 2016. The rate of fluoroquinolone-susceptible *Pseudomonas aeruginosa* isolated from urinary tract and respiratory tract infections improved during the period examined from 41.8–67.0% to 91.2–94.2%, and from 78.9–88.5% to 90.1–94.6%, respectively. Against *Acinetobacter* spp., the susceptibility rate of fluoroquinolones was almost constant at around 90%, but one multidrug-resistant isolate was detected in 2013. Overall, the susceptibility to fluoroquinolones was maintained over 20 years against tested bacteria except for MRSA and *E. coli*.

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1. Introduction

In recent years, as well as methicillin-resistant *Staphylococcus aureus* (MRSA) and penicillin-resistant *Streptococcus pneumoniae* (PRSP) [1], various drug-resistant bacteria have emerged, such as vancomycin-resistant enterococci (VRE) [2], extended-spectrum β -lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* [3], metallo- β -lactamase (MBL) producing Gram-negative

bacilli [4], carbapenem-resistant Enterobacteriaceae (CRE) [5], and multidrug-resistant *Acinetobacter* spp. (MDRA) or *Pseudomonas aeruginosa* (MDRP) [6,7]. The emergence of drug-resistant bacteria is now a worldwide problem, but development of new antibacterial agents is lagging behind. Under such conditions, the WHO warned that antimicrobial-resistance (AMR) was a big problem for international society and adopted global action plan for AMR in May 2015. With the proposal of the WHO, a national action plan on AMR was also settled on in Japan in April 2016 and continuous monitoring of AMR and drug usage is one of the six important goals [8].

The levofloxacin surveillance group started to understand the trends of drug-resistant bacteria in Japan in 1994 just after levofloxacin (LVFX) application. Fluoroquinolones (FQs) exhibit potent

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activity against Gram-positive and Gram-negative bacteria and good penetration into tissues in patients. The advantages led to a worldwide use of FQs, but resistant strains have appeared despite a low resistance rate being expected because of their mechanism of action, inhibition of DNA synthesis. Thus, we continued the surveillance study over 20 years and have already reported on the susceptibility trends from 1994 to 2002, especially for LVFX, which is the most popular FQ in the world [9]. A newer FQ, sitafloxacin, which was developed to beat FQ-resistant bacteria and approved in 2008 in Japan, was added as a test drug after 2002. In this manuscript we describe the results from 1994 to 2016 and survey drug resistance trends.

2. Materials and methods

This study was done as part of a post-marketing surveillance of LVFX according to the rules of the Ministry of Health and Labor of Japan.

2.1. Bacteria

Nationwide antibacterial susceptibility studies were performed every 2 or 3 years for the period between 1994 and 2016. Overall, 54,933 clinical isolates included *S. aureus* (methicillin-susceptible and methicillin-resistant), *S. pneumoniae*, *S. pyogenes*, *E. coli*, *K. pneumoniae*, *Salmonella* spp., *P. aeruginosa* isolated from urinary tract infections (UTI) or respiratory tract infections (RTI), *H. influenzae*, and *Acinetobacter* spp., were collected from 24 to 77 geographically diverse medical centers in Japan (Table 1). The geographic distribution was almost constant although the number of medical centers varied over the study period. Clinical isolates were obtained and identified from materials (eg, sputum, throat swab, urine, feces, and blood) collected from ambulatory and hospitalized patients suffered with various infectious diseases. All isolates were identified at a central laboratory (BML Inc., Saitama, Japan) for confirming the initial identification done in each center before susceptibility testing.

2.2. In vitro antibacterial susceptibility testing

The activity of various antibacterial agents against these clinical isolates was determined using the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) document [10]. The antimicrobial agents tested were as follows: levofloxacin (LVFX), ciprofloxacin (CPFX), sitafloxacin (STFX), benzylpenicillin (PCG), ampicillin (ABPC), clavulanic acid/amoxicillin (CVA/AMPC), sulbactam/ampicillin (SBT/ABPC), piperacillin (PIPC), oxacillin (MPIPC), cefaclor (CCL), cefotiam (CTM), cefdinir (CFDN),

ceftazidime (CAZ), cefotaxime (CTX), ceftriaxone (CTR), ceftiofime (CPR), meropenem (MEPM), imipenem (IPM), aztreonam (AZT), chloramphenicol (CP), minocycline (MINO), clarithromycin (CAM), vancomycin (VCM), daptomycin (DAP), sulfamethoxazole/trimethoprim (ST), gentamicin (GM), and amikacin (AMK). Quality control was performed by using control strains recommended by CLSI and all results showing discrepancies were repeated. The minimum inhibitory concentration (MIC) data were interpreted according to the breakpoints recommended by CLSI M100-S27 [11] or The European Committee on Antimicrobial Susceptibility Testing (EUCAST) Version 7.1 [12]. When the breakpoint was not indicated in the above two guidelines, interpretive criteria with similar features were used and the breakpoints are listed in the Tables 2 and 3 and 5–7.

The isolation frequency of community-acquired MRSA (CA-MRSA) was estimated by the MIC pattern (LVFX MIC \leq 1 μ g/mL, MINO \leq 4 μ g/mL, and CAM \leq 2 μ g/mL) [13].

2.3. Determination of amino acid alteration in quinolone resistance determining region (QRDR) of DNA gyrase and topoisomerase IV

Amino acid substitutions at major sites in QRDR were determined for *S. pneumoniae*, *S. pyogenes*, *E. coli*, *K. pneumoniae* and *H. influenzae* by direct sequencing from 2007 to 2013 [14], and by draft whole-genome sequencing (WGS) in 2016. We used a Nextera XT DNA library preparation kit (Illumina, Inc., CA, USA) to prepare DNA libraries for WGS. Libraries were sequenced on the Illumina MiSeq for 600 cycles (300-bp paired-end reads). Draft genomes (contigs) were obtained using the SPAdes v3.8.1 [15]. Nucleotide alignment of *gyrA*, *gyrB*, *parC*, and *parE* was performed using BLASTn [16] with reference to the following sequence: *S. pneumoniae* ATCC700669 (accession no. NC_011900); *S. pyogenes* M1 (NC_002737.2), *E. coli* MG1655 (U00096), *K. pneumoniae* ATCC BAA-2146 (NZ_CP006659), *H. influenzae* NCTC8143 (NZ_LN831035). The strains examined were selected randomly at 20% (at least 20 isolates) of each MIC group from LVFX-susceptible strains, and 10 isolates or all isolates if less than 10 were selected from LVFX-intermediate and -resistant strains for QRDR detection, basically. For *S. pneumoniae*, the major sites examined were Ser81 and Glu85 in *GyrA*, Asp435 and Glu474 in *GyrB*, Ser79 and Asp83 in *ParC*, and Asp435 and Glu474 in *ParE*. The substitutions at Ser81 and Glu85 in *GyrA*, and at Ser79 and Asp83 in *ParC* for *S. pyogenes*, at Ser83 and Asp87 in *GyrA*, and at Ser80 and Glu84 in *ParC* for *E. coli*, at Ser83 and Asp87 in *GyrA*, and at Ser80 in *ParC* for *K. pneumoniae*, at Ser84 and Asp88 in *GyrA*, and at Ser84 and Glu88 in *ParC* for *H. influenzae* were also determined.

Table 1

Number of clinical isolates in each study year.

Bacteria	Study year										Total
	1994	1996	1998	2000	2002	2004	2007	2010	2013	2016	
Methicillin-susceptible <i>S. aureus</i> (MSSA)	355	332	361	515	706	1126	736	745	725	676	6277
Methicillin-resistant <i>S. aureus</i> (MRSA)	358	347	399	548	700	1169	744	719	665	616	6265
<i>S. pneumoniae</i>	253	286	291	432	598	1010	677	661	599	565	5372
<i>S. pyogenes</i>	–	178	170	331	368	676	509	434	384	366	3416
<i>E. coli</i>	387	357	363	504	696	1105	743	741	712	669	6277
<i>K. pneumoniae</i>	366	343	319	449	630	1010	663	678	552	499	5509
<i>Salmonella</i> spp.	107	154	99	165	186	320	210	194	123	106	1664
<i>P. aeruginosa</i> from UTI	306	295	219	392	503	835	589	609	559	500	4807
<i>P. aeruginosa</i> from RTI	294	322	294	426	592	1049	673	660	616	578	5504
<i>H. influenzae</i>	292	315	295	442	627	1051	675	660	620	544	5521
<i>Acinetobacter</i> spp.	–	271	215	392	474	834	598	577	512	448	4321
Total	2718	3200	3025	4596	6080	10185	6817	6678	6067	5567	54933
Number of centers	24	25	26	37	52	77	72	72	69	65	100 ^a

^a Real total number excluding duplication.

Table 2
Percent changes in antimicrobial resistance rate during 1994–2016 for *S. aureus*.

Bacteria	Antimicrobial Agent	Susceptibility (%)	Breakpoint	1994	1996	1998	2000	2002	2004	2007	2010	2013	2016
Methicillin-susceptible <i>S. aureus</i> (MSSA)	LVFX	Susceptible	≤ 1	96.9	97.0	95.8	95.9	94.3	94.5	93.2	91.4	89.9	87.1
		Intermediate	2	0.6	0.9	–	0.6	1.0	1.7	1.0	0.3	1.8	1.3
		Resistant	≥ 4	2.5	2.1	4.2	3.5	4.7	3.8	5.8	8.3	8.3	11.5
	CPFX	Susceptible	≤ 1	95.2	91.3	92.0	93.0	91.8	92.4	90.2	88.5	87.0	83.0
		Intermediate	2	2.0	5.1	3.3	2.3	1.8	1.7	2.3	2.4	1.9	3.7
		Resistant	≥ 4	2.8	3.6	4.7	4.7	6.4	6.0	7.5	9.1	11.0	13.3
	STFX	Susceptible	≤ 1	–	–	–	–	99.6	99.2	99.3	99.1	99.3	99.1
		Intermediate	2	–	–	–	–	0.0	0.1	0.4	0.3	0.3	0.3
		Resistant	≥ 4	–	–	–	–	0.4	0.7	0.3	0.7	0.4	0.6
	ABPC	Susceptible	≤ 0.25	30.7	35.8	33.2	37.7	39.4	43.3	39.7	43.2	48.8	49.9
		Resistant	≥ 0.5	69.3	64.2	66.8	62.3	60.6	56.7	60.3	56.8	51.2	50.1
	CVA/AMPC	Susceptible	≤ 4	98.6	100.0	99.7	100.0	99.6	99.8	100.0	100.0	100.0	–
		Resistant	≥ 8	1.4	0.0	0.3	0.0	0.4	0.2	0.0	0.0	0.0	–
	SBT/ABPC	Susceptible	≤ 2	–	–	–	–	–	–	–	–	–	–
		Intermediate	4	–	–	–	–	–	–	–	–	–	99.3
		Resistant	≥ 8	–	–	–	–	–	–	–	–	–	0.7
	MINO	Susceptible	≤ 4	98.9	98.5	99.4	99.6	99.4	98.7	99.5	98.9	99.3	99.0
		Intermediate	8	0.0	0.0	0.3	0.4	0.0	0.4	0.4	0.4	0.3	0.9
		Resistant	≥ 16	1.1	1.5	0.3	0.0	0.6	0.9	0.1	0.7	0.4	0.1
	CAM	Susceptible	≤ 2	91.8	89.5	88.6	90.7	87.3	83.1	76.4	75.3	74.3	74.7
		Intermediate	4	0.3	0.6	0.8	2.1	4.1	2.7	1.2	0.4	1.1	3.6
		Resistant	≥ 8	7.9	9.9	10.5	7.2	8.6	14.2	22.4	24.3	24.6	21.7
	MPIPC	Susceptible	≤ 2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		Resistant	≥ 4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
VCM	Susceptible	≤ 2	100.0	99.7	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.6	
	Intermediate	4–8	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	
	Resistant	≥ 16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
DAP	Susceptible	≤ 1	–	–	–	–	–	–	–	–	–	99.9	
	Resistant	≥ 2	–	–	–	–	–	–	–	–	–	0.1	
CCL	Susceptible	≤ 8	100.0	96.1	99.4	100.0	99.6	99.8	99.9	99.9	99.7	100.0	
	Intermediate	16	0.0	3.0	0.3	0.0	0.1	0.1	0.1	0.1	0.3	0.0	
	Resistant	≥ 32	0.0	0.9	0.3	0.0	0.3	0.1	0.0	0.0	0.0	0.0	
CTM	Susceptible	≤ 1	91.3	99.1	99.2	99.2	99.3	99.5	94.8	93.7	97.8	99.6	
	Intermediate	2	0.8	0.3	0.3	0.8	0.4	0.4	4.9	6.3	2.2	0.4	
	Resistant	≥ 4	7.9	0.6	0.6	0.0	0.3	0.1	0.3	0.0	0.0	0.0	
Methicillin-resistant <i>S. aureus</i> (MRSA)	LVFX	Susceptible	≤ 1	18.2	9.5	7.8	6.0	7.3	8.3	5.8	11.0	17.0	15.4
		Intermediate	2	0.6	3.2	9.5	6.6	6.9	7.3	0.7	1.5	0.9	0.2
		Resistant	≥ 4	81.3	87.3	82.7	87.4	85.9	84.4	93.5	87.5	82.1	84.4
CPFX	Susceptible	≤ 1	13.1	6.3	6.0	5.1	7.4	7.6	5.6	10.6	15.8	14.6	
	Intermediate	2	2.0	2.9	1.5	0.9	0.1	0.3	0.1	0.3	1.1	0.5	
	Resistant	≥ 4	84.9	90.8	92.5	94.0	92.4	92.0	94.2	89.2	83.2	84.9	
STFX	Susceptible	≤ 1	–	–	–	–	76.9	63.7	55.2	51.6	55.3	69.3	
	Intermediate	2	–	–	–	–	6.3	6.1	10.8	9.3	8.1	4.7	
	Resistant	≥ 4	–	–	–	–	16.9	30.2	34.0	39.1	36.5	26.0	
ABPC	Susceptible	≤ 0.25	0.0	0.0	0.5	0.0	0.0	0.1	0.0	0.1	0.0	0.2	
	Resistant	≥ 0.5	100.0	100.0	99.5	100.0	100.0	99.9	100.0	99.9	100.0	99.8	
CVA/AMPC	Susceptible	≤ 4	3.1	1.7	1.8	3.1	2.6	3.3	3.1	7.2	22.9	–	
	Resistant	≥ 8	96.9	98.3	98.2	96.9	97.4	96.7	96.9	92.8	77.1	–	
SBT/ABPC	Susceptible	≤ 2	–	–	–	–	–	–	–	–	–	–	
	Intermediate	4	–	–	–	–	–	–	–	–	–	4.5	
	Resistant	≥ 8	–	–	–	–	–	–	–	–	–	18.0	
MINO	Susceptible	≤ 4	41.6	32.0	36.1	35.6	44.1	43.8	35.2	37.8	55.6	67.4	
	Intermediate	8	3.6	6.1	20.6	21.7	32.0	28.3	36.7	16.7	12.2	27.1	
	Resistant	≥ 16	54.7	62.0	43.4	42.7	23.9	27.9	28.1	45.5	32.2	5.5	
CAM	Susceptible	≤ 2	4.7	4.0	3.8	5.1	5.0	6.4	4.4	5.7	14.0	18.7	
	Intermediate	4	0.3	0.6	0.5	1.5	1.6	1.6	0.1	0.1	0.5	5.4	
	Resistant	≥ 8	95.0	95.4	95.7	93.4	93.4	92.0	95.4	94.2	85.6	76.0	
MPIPC	Susceptible	≤ 2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	Resistant	≥ 4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
VCM	Susceptible	≤ 2	100.0	99.4	99.7	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
	Intermediate	4–8	0.0	0.6	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	Resistant	≥ 16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
DAP	Susceptible	≤ 1	–	–	–	–	–	–	–	–	–	100.0	
	Resistant	≥ 2	–	–	–	–	–	–	–	–	–	0.0	
CCL	Susceptible	≤ 8	100.0	0.0	0.5	1.1	1.7	1.4	1.9	4.5	9.6	12.3	
	Intermediate	16	0.0	0.3	2.8	2.6	3.7	3.7	1.7	5.8	12.5	17.4	
	Resistant	≥ 32	0.0	99.7	96.7	96.4	94.6	95.0	96.4	89.7	77.9	70.3	
CTM	Susceptible	≤ 1	0.3	0.0	0.8	0.7	1.0	1.5	0.3	0.4	1.7	1.5	
	Intermediate	2	2.5	0.6	5.3	4.0	8.1	8.0	3.2	8.5	18.5	37.3	
	Resistant	≥ 4	97.2	99.4	94.0	95.3	90.9	90.6	96.5	91.1	79.8	61.2	

LVFX, levofloxacin; CPFX, ciprofloxacin; STFX, sitafloxacin; ABPC, ampicillin; CVA/AMPC, clavulanic acid/amoxicillin; SBT/ABPC, sulbactam/ampicillin; MINO, minocycline; CAM, clarithromycin; MPIPC, oxacillin; VCM, vancomycin; CCL, cefaclor; CTM, cefotiam; DAP, daptomycin.

Table 3
Percent changes in antimicrobial resistance rate during 1994–2016 for streptococci.

Bacteria	Antimicrobial Agent	Susceptibility (%)	Breakpoint	1994	1996	1998	2000	2002	2004	2007	2010	2013	2016	
<i>S. pneumoniae</i>	LVFX	Susceptible	≤2	97.2	99.3	99.0	98.4	98.0	99.2	98.8	98.5	97.8	97.3	
		Intermediate	4	0.4	0.3	0.3	0.5	0.3	0.5	0.1	0.6	0.5	0.2	
		Resistant	≥8	2.4	0.3	0.7	1.2	1.7	0.3	1.0	0.9	1.7	2.5	
	CPFX	Susceptible	≤1	69.2	93.7	90.7	95.4	95.5	96.7	87.0	80.2	74.3	89.4	
		Intermediate	2	23.7	4.5	6.9	3.0	1.5	1.7	9.2	17.7	21.0	7.6	
		Resistant	≥4	7.1	1.7	2.4	1.6	3.0	1.6	3.8	2.1	4.7	3.0	
	STFX	Susceptible	≤1	–	–	–	–	100.0	100.0	100.0	100.0	100.0	100.0	99.8
		Intermediate	2	–	–	–	–	0.0	0.0	0.0	0.0	0.0	0.0	0.2
		Resistant	≥4	–	–	–	–	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	CVA/AMPC	Susceptible	≤2	99.2	99.7	98.6	99.3	97.5	96.2	99.3	99.5	98.2	–	
		Intermediate	4	0.4	0.3	1.4	0.2	2.2	3.2	0.6	0.3	1.2	–	
		Resistant	≥8	0.4	0.0	0.0	0.5	0.3	0.6	0.1	0.2	0.7	–	
	SBT/ABPC	Susceptible	≤0.5	–	–	–	–	–	–	–	–	–	–	75.4
		Intermediate	1	–	–	–	–	–	–	–	–	–	–	8.3
		Resistant	≥2	–	–	–	–	–	–	–	–	–	–	16.3
	CCL	Susceptible	≤1	38.7	53.8	59.1	50.7	44.3	33.2	51.0	44.3	58.8	59.1	
		Intermediate	2	23.7	8.4	8.2	5.3	13.2	24.0	5.3	7.4	4.8	5.0	
		Resistant	≥4	37.5	37.8	32.6	44.0	42.5	42.9	43.7	48.3	36.4	35.9	
	CTM	Susceptible	≤0.5	75.5	66.4	65.6	63.9	60.9	46.0	61.3	57.6	71.0	72.4	
		Intermediate	1	5.9	4.9	15.1	12.5	16.6	12.9	12.1	13.8	8.5	9.7	
		Resistant	≥2	18.6	28.7	19.2	23.6	22.6	41.1	26.6	28.6	20.5	17.9	
	CTRX	Susceptible	≤1	–	–	–	–	–	93.4	97.3	97.4	97.2	97.9	
		Intermediate	2	–	–	–	–	–	5.4	2.2	2.0	1.3	1.1	
		Resistant	≥4	–	–	–	–	–	1.2	0.4	0.6	1.5	1.1	
	MINO	Susceptible	≤2	28.5	29.4	30.9	27.5	26.6	28.7	40.2	32.5	37.7	57.7	
		Intermediate	4	3.6	17.8	25.8	23.6	19.6	32.4	30.7	31.2	28.7	28.8	
		Resistant	≥8	68.0	52.8	43.3	48.8	53.8	38.9	29.1	36.3	33.6	13.5	
	CAM	Susceptible	≤0.25	55.3	37.8	46.7	32.9	53.5	33.7	19.4	15.9	15.2	20.0	
		Intermediate	0.5	2.4	6.3	17.2	14.8	10.4	9.5	5.9	11.6	9.3	7.1	
		Resistant	≥1	42.3	55.9	36.1	52.3	36.1	56.8	74.7	72.5	75.5	72.9	
	PCG	Susceptible	≤0.06	–	52.8	54.6	49.3	49.0	44.8	52.3	48.0	57.6	56.8	
		Intermediate	0.12–1	–	33.6	40.2	45.8	44.0	41.4	40.2	43.4	36.7	36.8	
		Resistant	≥2	–	13.6	5.2	4.9	7.0	13.9	7.5	8.6	5.7	6.4	
	<i>S. pyogenes</i>	LVFX	Susceptible	≤2	–	100.0	100.0	100.0	99.5	99.4	98.4	98.2	96.1	95.9
			Intermediate	4	–	0.0	0.0	0.0	0.3	0.0	0.4	0.5	1.0	1.4
			Resistant	≥8	–	0.0	0.0	0.0	0.3	0.6	1.2	1.4	2.9	2.7
CPFX		Susceptible	≤1	–	92.1	92.9	93.7	98.9	89.1	86.8	81.6	78.9	82.0	
		Intermediate	2	–	7.3	7.1	6.3	0.5	10.4	11.6	15.0	14.3	13.7	
		Resistant	≥4	–	0.6	0.0	0.0	0.5	0.6	1.6	3.5	6.8	4.4	
STFX		Susceptible	≤1	–	–	–	–	100.0	100.0	100.0	100.0	100.0	100.0	
		Intermediate	2	–	–	–	–	0.0	0.0	0.0	0.0	0.0	0.0	
		Resistant	≥4	–	–	–	–	0.0	0.0	0.0	0.0	0.0	0.0	
CVA/AMPC		Susceptible	≤8	–	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	–	
		Intermediate	16	–	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	–	
		Resistant	≥32	–	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	–	
SBT/ABPC		Susceptible	≤0.25	–	–	–	–	–	–	–	–	–	–	100.0
		Intermediate	0.5	–	–	–	–	–	–	–	–	–	–	0.0
		Resistant	≥1	–	–	–	–	–	–	–	–	–	–	0.0
CCL		Susceptible	≤8	–	99.4	99.4	100.0	99.7	100.0	100.0	100.0	100.0	100.0	
		Intermediate	16	–	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
		Resistant	≥32	–	0.6	0.6	0.0	0.3	0.0	0.0	0.0	0.0	0.0	
CTM		Susceptible	≤1	–	100.0	99.4	100.0	99.7	99.9	99.8	99.5	100.0	100.0	
		Intermediate	2	–	0.0	0.0	0.0	0.0	0.1	0.2	0.5	0.0	0.0	
		Resistant	≥4	–	0.0	0.6	0.0	0.3	0.0	0.0	0.0	0.0	0.0	
CTRX		Susceptible	≤0.5	–	–	–	–	–	100.0	100.0	100.0	100.0	100.0	
		Intermediate	1	–	–	–	–	–	0.0	0.0	0.0	0.0	0.0	
		Resistant	≥1	–	–	–	–	–	0.0	0.0	0.0	0.0	0.0	
MINO		Susceptible	≤2	–	75.3	91.8	87.0	75.8	94.8	90.4	84.8	87.2	93.7	
		Intermediate	4	–	6.2	4.1	8.8	5.4	1.6	5.7	5.1	4.4	1.4	
		Resistant	≥8	–	18.5	4.1	4.2	18.8	3.6	3.9	10.1	8.3	4.9	
CAM		Susceptible	≤0.25	–	94.4	93.5	92.4	91.0	85.2	76.8	56.0	64.6	68.0	
		Intermediate	0.5	–	3.4	0.6	0.6	0.8	1.3	1.0	1.4	1.0	1.4	
		Resistant	≥1	–	2.2	5.9	6.9	8.2	13.5	22.2	42.6	34.4	30.6	
PCG		Susceptible	≤0.12	–	100.0	99.4	99.7	99.7	99.9	100.0	100.0	100.0	100.0	
		Intermediate	0.25	–	0.0	0.6	0.3	0.3	0.1	0.0	0.0	0.0	0.0	
		Resistant	≥1	–	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	

LVFX, levofloxacin; CPFX, ciprofloxacin; STFX, sitafloxacin; CVA/AMPC, clavulanic acid/amoxicillin; SBT/ABPC, sulbactam/ampicillin; CCL, cefaclor; CTM, cefotiam; CTRX, ceftriaxone; MINO, minocycline; CAM, clarithromycin; PCG, penicillin.

3. Results

3.1. *Staphylococcus aureus*

The susceptibilities of *S. aureus* to the antimicrobials tested are shown in Table 2. The resistance rate for methicillin-susceptible

S. aureus (MSSA) to LVFX and CPFX rose gradually during the study period, and STFX showed a lower resistance rate during 2002 and 2016. By contrast, the resistance rate of MRSA to LVFX and CPFX remained high at 81.9–94.9%, and the resistance rate to STFX was relatively low at 23.2–48.4%. However, the susceptibility rate to FQs was gradually increasing after 2007. MINO maintained a high

Table 4
Analysis of strains with amino acid substitution in quinolone resistance determining region during 2007–2016.

Organism	Number of amino acid substitution	Number of strains														
		LVFX MIC ($\mu\text{g/mL}$)														
		0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32 ^b	64
<i>S. pneumoniae</i> (860) ^a	0						5	17	149	560	28	2	2	4	0	1
	1						0	0	3	28	10	2	1	0	0	0
	2						0	0	1	3	0	4	12	5	0	0
	3						0	0	0	0	1	0	0	2	1	0
	4						0	0	0	0	0	0	0	0	0	0
<i>S. pyogenes</i> (311) ^a	0						13	49	99	31	7	0	0	0	0	
	1						2	1	1	17	40	6	2	3	1	
	2						0	0	0	0	3	7	4	20	0	
	3						0	0	0	0	0	0	0	0	0	
	4						0	0	0	0	0	0	0	0	0	
<i>E. coli</i> (656) ^a	0	5	31	57	88	48	16	3	4	1	0	0	0	0	0	0
	1	0	0	0	0	1	36	94	64	28	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	10	19	1	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	1	5	18	6	4	6	3
	4	0	0	0	0	0	0	0	0	0	4	12	24	26	24	14
<i>K. pneumoniae</i> (529) ^a	0	1	3	26	71	142	36	29	45	43	9	11	5	3	2	0
	1	1	0	1	3	0	4	10	1	4	4	4	4	1	1	0
	2	0	0	0	0	0	0	0	0	0	1	1	3	5	3	1
	3	0	0	0	0	0	0	0	0	0	0	0	0	6	2	3
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>H. influenzae</i> (444) ^a	0	5	48	228	48	6	4	1	2	1	0	0	0	0	0	
	1	0	0	8	2	16	26	1	1	1	0	0	3	1	0	
	2	0	0	1	1	0	2	0	16	2	0	0	1	0	0	
	3	0	0	0	1	0	0	0	0	0	1	0	4	0	1	
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

^a Total number of strains QRDR examined during 2007–2016.

^b >16 $\mu\text{g/mL}$ for *H. influenzae*.

susceptibility rate against MSSA, but CAM showed a falling susceptibility rate. On the other hand, the susceptibility rate to MINO and CAM against MRSA increased after 2010, reaching 67.4% and 18.7% in 2016. VCM-intermediate resistant strains (MIC = 4 $\mu\text{g/mL}$) in MSSA were identified at 0.3% (1 isolate) in 1996 and 0.4% (3 isolates) in 2013, and in MRSA at 0.6% (2 isolates) in 1996 and 0.3% (1 isolate) in 1998, and no VCM-resistant *S. aureus* was observed. Although VCM-intermediate resistant MRSA was not detected after 2000, one DAP-resistant strain (MIC = 4 $\mu\text{g/mL}$) was isolated in 2016.

The isolation frequency of community-acquired MRSA (CA-MRSA) estimated by MIC pattern (LVFX MIC \leq 1 $\mu\text{g/mL}$, MINO \leq 4 $\mu\text{g/mL}$, and CAM \leq 2 $\mu\text{g/mL}$) was 2.2% (8 isolates) in 1994, 1.4% (5 isolates) in 1996, 2.0% (8 isolates) in 1998, 1.1% (6 isolates) in 2000, 2.4% (17 isolates) in 2002, 2.2% (26 isolates) in 2004, 2.3% (17 isolates) in 2007, 4.6% (33 isolates) in 2010, 9.3% (62 isolates) in 2013, and 7.3% (45 isolates) in 2016. One third to 3/4 of the CA-MRSA was isolated from inpatients in each year and 1 isolate in 2004 showed IPM resistance.

3.2. Streptococci

The percentage changes in antimicrobial resistance rate for streptococci are shown in Table 3. The percentage of penicillin-susceptible *S. pneumoniae* (PSSP) and PRSP including intermediate strains over 20 years was nearly constant at about 50%. The range of resistance rate of *S. pneumoniae* to LVFX and STFX remained low, however, an LVFX-resistant strain with MIC of 64 $\mu\text{g/mL}$ was isolated for the first time in 2016, which showed STFX-intermediate resistance (MIC = 2 $\mu\text{g/mL}$). Amino acid substitutions at the major sites in QRDR were determined after 2007 and the number of strains with amino acid substitution(s) is listed in Table 4. Among 28 LVFX-resistant strains examined, 7 strains including the strain with LVFX MIC of 64 $\mu\text{g/mL}$ showed no major alteration in QRDR, 1 strain showed single substitution in ParC, 14 and 3 strains showed double substitutions in GyrA and ParC and

GyrA and ParE, respectively, 2 strains showed triple substitutions in GyrA, ParC and ParE, and 1 strain with LVFX MIC of 32 $\mu\text{g/mL}$ showed double substitutions in GyrB and a single substitution in ParC. The range of CFX-resistance rate rose and fell over 20 years, without showing an upward trend as a whole. The susceptibility rate to CAM was lowered but the susceptibility rate to CTRX remained high at 93.4–97.9% during the test period.

Regarding *S. pyogenes*, a high susceptibility rate to LVFX and STFX was observed during the study period. However, the LVFX-intermediate and -resistant rate gradually increased over 20 years. The QRDR alterations in GyrA and/or ParC were detected in all LVFX-resistant strains and ParC alteration was detected in 60 of 98 LVFX-susceptible strains tested with MIC of 1 and 2 $\mu\text{g/mL}$ (Table 4). MINO showed a recovery of its susceptibility to 93.7% in 2016, but CAM-intermediate and -resistant rate increased from 5.6% in 1994 to 32.0% in 2016, although the resistance rate in 2016 was lower than the rate in 2010 and 2013.

3.3. Enterobacteriaceae

Table 5 listed the susceptibility of Enterobacteriaceae to the antimicrobial agents tested. The resistance rate including “intermediate” and “resistant” of *E. coli* to LVFX and CFX rose from 2.1–2.4% in 1994 to 36.6–37.6% in 2016 and the resistance rate (“intermediate” and “resistant”) to STFX showed an increase from 3.0% in 2002 to 12.7% in 2016. Three or four QRDR alterations in GyrA and ParC were detected in all LVFX-resistant and -intermediate strains (Table 4). Two, three, or four alterations were detected in all strains with LVFX MIC of 2 $\mu\text{g/mL}$, while these strains were defined as LVFX-susceptible, and at least one-alteration was observed in 252/276 LVFX-susceptible strains with MIC of 0.12–1 $\mu\text{g/mL}$. The resistance rate (“intermediate” and “resistant”) to CCL, CTM, and CFDN also increased during the study period. One IPM-intermediate resistant strain (MIC = 8 $\mu\text{g/mL}$) was detected in 2016.

Table 5
Percent changes in antimicrobial resistance rate during 1994–2016 for Enterobacteriaceae.

Bacteria	Antimicrobial Agent	Susceptibility (%)	Breakpoint	1994	1996	1998	2000	2002	2004	2007	2010	2013	2016	
<i>E. coli</i>	LVFX	Susceptible	≥2	97.9	97.5	96.7	91.9	88.2	81.2	73.8	70.7	65.6	63.4	
		Intermediate	4	0.5	1.4	0.8	2.0	3.2	2.0	2.8	2.2	2.5	2.4	
		Resistant	≧8	1.6	1.1	2.5	6.2	8.6	16.8	23.4	27.1	31.9	34.2	
	CPFX	Susceptible	≥1	97.7	97.5	96.4	90.9	87.5	80.9	73.8	70.2	65.2	62.3	
		Intermediate	2	0.3	0.0	0.3	0.4	0.4	0.3	0.4	0.3	0.1	0.4	
		Resistant	≧4	2.1	2.5	3.3	8.7	12.1	18.8	25.8	29.6	34.7	37.2	
	STFX	Susceptible	≥1	–	–	–	–	97.0	95.1	91.8	91.9	85.5	87.3	
		Intermediate	2	–	–	–	–	1.9	3.8	6.5	6.1	11.2	9.4	
		Resistant	≧4	–	–	–	–	1.1	1.1	1.7	2.0	3.2	3.3	
	ABPC	Susceptible	≥8	67.4	68.9	72.7	69.0	68.7	64.3	59.4	55.6	51.3	49.6	
		Intermediate	16	0.8	0.8	1.4	0.2	0.6	0.5	1.7	1.5	2.4	1.8	
		Resistant	≧32	31.8	30.3	25.9	30.8	30.7	35.2	38.9	42.9	46.3	48.6	
	CVA/AMPC	Susceptible	≥8	77.0	82.6	73.8	73.2	60.1	71.4	76.6	79.5	81.2	–	
		Intermediate	16	6.7	6.4	12.7	10.1	20.4	18.3	15.9	12.0	12.6	–	
		Resistant	≧32	16.3	10.9	13.5	16.7	19.5	10.3	7.5	8.5	6.2	–	
	SBT/ABPC	Susceptible	≥8	–	–	–	–	–	–	–	–	–	–	65.2
		Intermediate	16	–	–	–	–	–	–	–	–	–	–	19.3
		Resistant	≧32	–	–	–	–	–	–	–	–	–	–	15.5
	CCL	Susceptible	≥8	81.7	86.8	90.9	89.9	91.8	90.3	84.7	80.4	74.2	73.4	
		Intermediate	16	3.1	3.6	0.8	1.0	3.0	0.9	1.6	2.4	2.4	2.1	
		Resistant	≧32	15.2	9.5	8.3	9.1	5.2	8.8	13.7	17.1	23.5	24.5	
	CTM	Susceptible	≥1	92.5	94.7	97.2	97.6	95.4	94.3	87.2	84.8	78.2	75.6	
		Intermediate	2	1.8	2.0	2.5	0.8	1.1	1.0	1.5	2.4	1.5	0.6	
		Resistant	≧4	5.7	3.4	0.3	1.6	3.4	4.7	11.3	12.8	20.2	23.8	
	CFDN	Susceptible	≥1	86.6	91.3	91.5	90.5	92.0	91.0	85.5	82.1	75.0	–	
		Intermediate	2	2.3	1.1	1.1	1.0	2.7	1.0	1.6	1.5	1.0	–	
		Resistant	≧4	11.1	7.6	7.4	8.5	5.3	8.1	12.9	16.5	24.0	–	
	IPM	Susceptible	≥1	–	–	–	–	–	100.0	100.0	100.0	100.0	99.9	
		Intermediate	2	–	–	–	–	–	0.0	0.0	0.0	0.0	0.0	
		Resistant	≧4	–	–	–	–	–	0.0	0.0	0.0	0.0	0.1	
	MINO	Susceptible	≥4	81.1	85.7	89.8	86.7	90.2	89.6	92.7	88.8	89.0	94.2	
		Intermediate	8	3.4	2.5	3.3	6.7	6.5	4.3	4.0	3.4	5.9	2.2	
		Resistant	≧16	15.5	11.8	6.9	6.5	3.3	6.1	3.2	7.8	5.1	3.6	
	ST	Susceptible	≥2	57.1	70.6	75.2	43.7	65.8	43.4	59.2	63.2	58.4	55.6	
		Resistant	≧4	42.9	29.4	24.8	56.3	34.2	56.6	40.8	36.8	41.6	44.4	
	CP	Susceptible	≥8	78.0	89.1	88.2	86.1	84.9	61.5	84.9	86.6	–	–	
		Intermediate	16	12.4	2.0	5.5	3.6	6.5	25.6	7.7	7.2	–	–	
		Resistant	≧32	9.6	9.0	6.3	10.3	8.6	12.9	7.4	6.2	–	–	
	<i>K. pneumoniae</i>	LVFX	Susceptible	≥2	99.7	100.0	98.1	99.1	98.4	98.8	98.0	97.5	95.8	95.8
			Intermediate	4	0.0	0.0	0.9	0.7	0.8	0.5	0.8	0.3	0.9	1.8
			Resistant	≧8	0.3	0.0	0.9	0.2	0.8	0.7	1.2	2.2	3.3	2.4
		CPFX	Susceptible	≥1	99.7	100.0	97.8	98.9	98.4	98.1	97.4	96.5	94.6	94.6
			Intermediate	2	0.0	0.0	0.9	0.4	0.5	0.9	0.8	1.0	1.4	1.0
			Resistant	≧4	0.3	0.0	1.3	0.7	1.1	1.0	1.8	2.5	4.0	4.4
		STFX	Susceptible	≥1	–	–	–	–	99.0	99.7	99.2	97.9	96.7	96.6
			Intermediate	2	–	–	–	–	0.6	0.0	0.6	0.6	2.0	1.8
			Resistant	≧4	–	–	–	–	0.3	0.3	0.2	1.5	1.3	1.6
		ABPC	Susceptible	≥8	9.8	11.1	17.9	17.8	13.0	18.2	4.8	5.2	7.4	7.4
Intermediate			16	21.6	29.4	37.6	39.0	35.2	39.3	17.3	16.4	21.4	22.6	
Resistant			≧32	68.6	59.5	44.5	43.2	51.7	42.5	77.8	78.5	71.2	69.9	
CVA/AMPC		Susceptible	≥8	94.3	94.8	95.9	97.1	92.2	95.9	94.0	94.0	93.1	–	
		Intermediate	16	1.6	1.7	1.9	1.6	4.1	2.2	3.8	3.2	4.7	–	
		Resistant	≧32	4.1	3.5	2.2	1.3	3.7	1.9	2.3	2.8	2.2	–	
SBT/ABPC		Susceptible	≥8	–	–	–	–	–	–	–	–	–	–	83.4
		Intermediate	16	–	–	–	–	–	–	–	–	–	–	6.4
		Resistant	≧32	–	–	–	–	–	–	–	–	–	–	10.2
CCL		Susceptible	≥8	94.8	93.9	97.2	98.4	97.1	97.0	95.0	93.5	90.4	91.8	
		Intermediate	16	0.0	0.6	0.0	0.2	0.6	0.3	0.3	0.4	0.4	0.6	
		Resistant	≧32	5.2	5.5	2.8	1.3	2.2	2.7	4.7	6.0	9.2	7.6	
CTM		Susceptible	≥1	98.1	94.8	96.9	98.4	97.0	96.8	92.9	92.8	88.8	90.2	
		Intermediate	2	0.8	0.9	0.3	0.4	1.0	0.4	1.8	1.3	2.2	1.8	
		Resistant	≧4	1.1	4.4	2.8	1.1	2.1	2.8	5.3	5.9	9.1	8.0	
CFDN		Susceptible	≥1	98.1	95.0	97.2	98.7	97.5	97.3	94.7	94.1	91.1	–	
		Intermediate	2	0.5	1.7	0.9	0.4	0.5	0.2	0.8	0.1	0.2	–	
		Resistant	≧4	1.4	3.2	1.9	0.9	2.1	2.5	4.5	5.8	8.7	–	
IPM		Susceptible	≥1	–	–	–	–	–	99.8	99.4	99.7	99.6	99.4	
		Intermediate	2	–	–	–	–	–	0.2	0.5	0.1	0.0	0.4	
		Resistant	≧4	–	–	–	–	–	0.0	0.2	0.1	0.4	0.2	
MINO		Susceptible	≥4	85.0	91.5	90.6	93.3	90.3	91.3	91.6	86.9	87.7	88.6	
		Intermediate	8	5.7	2.9	2.2	1.8	3.7	2.3	3.5	3.8	3.3	2.8	
		Resistant	≧16	9.3	5.5	7.2	4.9	6.0	6.4	5.0	9.3	9.1	8.6	
ST		Susceptible	≥2	23.2	54.2	43.6	12.7	56.5	19.4	29.1	22.6	23.6	14.8	
		Resistant	≧4	76.8	45.8	56.4	87.3	43.5	80.6	70.9	77.4	76.4	85.2	
CP		Susceptible	≥8	88.8	90.7	86.8	92.2	89.4	86.9	88.4	85.7	–	–	
		Intermediate	16	2.7	1.5	0.9	0.7	2.4	2.1	0.8	2.7	–	–	
		Resistant	≧32	8.5	7.9	12.2	7.1	8.3	11.0	10.9	11.7	–	–	

(continued on next page)

Table 5 (continued)

Bacteria	Antimicrobial Agent	Susceptibility (%)	Breakpoint	1994	1996	1998	2000	2002	2004	2007	2010	2013	2016
<i>Salmonella</i> spp.	LVFX	Susceptible	≤0.12	98.1	98.1	100.0	98.2	92.5	93.1	91.9	91.8	91.9	86.8
		Intermediate	0.25–1	1.9	1.9	0.0	1.8	7.5	5.9	7.6	8.2	7.3	10.4
		Resistant	≥2	0.0	0.0	0.0	0.0	0.0	0.9	0.5	0.0	0.8	2.8
	CPFX	Susceptible	≤0.06	98.1	98.1	100.0	98.2	96.2	92.2	91.9	90.2	91.9	86.8
		Intermediate	0.12–0.5	1.9	1.9	0.0	1.8	3.8	6.9	6.7	9.8	6.5	8.5
		Resistant	≥1	0.0	0.0	0.0	0.0	0.0	0.9	1.4	0.0	1.6	4.7
	STFX	Susceptible	≤0.06	–	–	–	–	99.5	99.1	94.8	97.4	96.7	92.5
		Intermediate	0.12–0.5	–	–	–	–	0.5	0.9	5.2	2.6	3.3	5.7
		Resistant	≥1	–	–	–	–	0.0	0.0	0.0	0.0	0.0	1.9
	ABPC	Susceptible	≤8	83.2	86.4	98.0	90.9	92.5	95.0	92.4	91.8	82.9	86.8
		Intermediate	16	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Resistant	≥32	16.8	13.0	2.0	9.1	7.5	5.0	7.6	8.2	17.1	13.2
	CVA/AMPC	Susceptible	≤8	85.0	92.9	99.0	93.9	93.5	96.3	95.2	96.4	99.2	–
		Intermediate	16	6.5	1.9	1.0	5.5	1.6	1.9	1.9	3.1	0.0	–
		Resistant	≥32	8.4	5.2	0.0	0.6	4.8	1.9	2.9	0.5	0.8	–
	SBT/ABPC	Susceptible	≤8	–	–	–	–	–	–	–	–	–	86.8
		Intermediate	16	–	–	–	–	–	–	–	–	–	8.5
		Resistant	≥32	–	–	–	–	–	–	–	–	–	4.7
	CCL	Susceptible	≤8	96.3	96.1	100.0	100.0	100.0	99.1	98.6	98.5	97.6	96.2
		Intermediate	16	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0
		Resistant	≥32	3.7	2.6	0.0	0.0	0.0	0.9	1.4	1.5	1.6	3.8
CTM	Susceptible	≤1	99.1	98.7	100.0	100.0	98.9	99.1	98.6	99.0	97.6	96.2	
	Intermediate	2	0.9	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.9	
	Resistant	≥4	0.0	0.6	0.0	0.0	1.1	0.9	1.4	1.0	1.6	2.8	
CFDN	Susceptible	≤1	100.0	98.7	100.0	100.0	98.9	99.1	98.6	98.5	98.4	–	
	Intermediate	2	0.0	0.6	0.0	0.0	0.5	0.0	0.0	0.0	0.0	–	
	Resistant	≥4	0.0	0.6	0.0	0.0	0.5	0.9	1.4	1.5	1.6	–	
MINO	Susceptible	≤4	52.3	77.9	89.9	91.5	95.7	94.7	92.4	94.8	87.0	90.6	
	Intermediate	8	16.8	5.2	1.0	5.5	0.5	1.6	1.0	1.0	1.6	3.8	
	Resistant	≥16	30.8	16.9	9.1	3.0	3.8	3.8	6.7	4.1	11.4	5.7	
ST	Susceptible	≤2	52.3	84.4	85.9	61.2	75.3	44.1	81.0	83.0	73.2	73.6	
	Resistant	≥4	47.7	15.6	14.1	38.8	24.7	55.9	19.0	17.0	26.8	26.4	
CP	Susceptible	≤8	79.4	92.2	100.0	95.8	92.5	59.7	94.3	91.8	–	–	
	Intermediate	16	1.9	1.3	0.0	0.6	4.3	35.9	1.9	1.5	–	–	
		Resistant	≥32	18.7	6.5	0.0	3.6	3.2	4.4	3.8	6.7	–	

LVFX, levofloxacin; CPFX, ciprofloxacin; STFX, sitafloxacin; ABPC, ampicillin; CVA/AMPC, clavulanic acid/amoxicillin; SBT/ABPC, sulbactam/ampicillin; CCL, cefaclor; CTM, cefotiam; CFDN, cefdinir; IPM, imipenem; MINO, minocycline; ST, sulfamethoxazole + trimethoprim; CP, chloramphenicol.

The resistance rate of *K. pneumoniae* to LVFX and CPFX increased slightly. As shown in Table 4, two or three QRDR alterations in GyrA and ParC were detected in 24/31 LVFX-resistant strains with LVFX MIC over 16 µg/mL and no alteration was observed in 16/28 isolates with LVFX MIC of 4 and 8 µg/mL, and in almost all LVFX-susceptible strains (45/46, 43/47 and 9/14 strains with LVFX MIC of 0.5, 1, and 2 µg/mL, respectively). IPM-resistant isolates were detected in 2007 and 2013 with MIC of 32 and 128 µg/mL, respectively. These 2 strains were susceptible to FQs, but resistant to cepheims, ST, and aminoglycosides.

For *Salmonella* spp., no resistant strain to LVFX, CPFX, and STFX was observed until 2002, 2002, and 2013, respectively. The FQ susceptibility percentage decreased slightly to 86.8–92.5% in 2016.

3.4. *P. aeruginosa*

The susceptibility patterns of *P. aeruginosa* are shown in Table 6. The susceptibility rate of *P. aeruginosa* isolated from UTI to all antimicrobial agents tested rose during the study period, and the susceptibility rate became almost the same as that of *P. aeruginosa* isolated from RTI in 2016, in contrast to the situation in 1994 in which the susceptibility rate against *P. aeruginosa* from UTI was much lower than that against *P. aeruginosa* from RTI. The susceptibility rate of *P. aeruginosa* from UTI to LVFX and CPFX increased to 91.2% in 2016, and GM and AMK also showed high activity, but IPM-intermediate and -resistant strains were isolated at 21.6% in 1994, and at 13.2% in 2016. Also, the susceptibility rate of *P. aeruginosa* isolated from RTI to all antimicrobial agents tested rose during the study period. FQ susceptibility rate was 90.1–94.6% in 2016.

The isolation frequency of multidrug-resistant *P. aeruginosa* (MDRP; CPFX ≥ 4 µg/mL, IPM ≥ 16 µg/mL and AMK ≥ 32 µg/mL) from UTI and RTI was listed each in the bottom line of Table 6. The MDRP rate of *P. aeruginosa* isolated from UTI decreased from 4.2% in 1994 to 0.8% in 2016, while the rate showed low (0.0–1.4%) in *P. aeruginosa* isolated from RTI during the study period.

3.5. *H. influenzae*

As shown in Table 7, FQs showed high activity with a susceptibility percentage of 99.1–100.0% during the study period. Twelve LVFX-resistant strains were isolated during the study period, and among these, 2 and 1 strains with LVFX MIC of 8 and >16 µg/mL, respectively, were isolated in 2016. These 3 strains also showed resistance to CPFX, ABPC, CCL (2 strains), and CAM (1 intermediate and 1 resistant strains), but were susceptible to STFX, CTX, IPM and MINO, and harbored double or triple amino acid substitutions in GyrA and ParC. One, two, or three amino acid substitutions were observed in 17/19, 3/4, and 1/1 strains with LVFX MIC of 0.5, 1, and 2 µg/mL, respectively (Table 4). The isolation frequency of β-lactamase-negative ABPC-susceptible *H. influenzae* (BLNAS), β-lactamase-negative ABPC-resistant *H. influenzae* (BLNAR), β-lactamase-positive ABPC-resistant *H. influenzae* (BLPAR), and β-lactamase-positive ABPC/CVA-resistant *H. influenzae* (BLPACR) was determined after 2004. The frequency of BLNAS lowered from 55.3% in 2004 to 32.2% in 2016. The isolation frequency of BLNAR, and BLPAR was 40.0% and 4.7% in 2004, 50.1% and 7.1% in 2007, 57.9% and 10.0% in 2010, 57.1% and 7.3% in 2013, and 55.9% and 11.9% in 2016, respectively. Among BLPAR, CVA/AMPC or SBT/ABPC-resistant strains (BLPACR) were detected 16.3% (0.8% of

Table 6
Percent changes in antimicrobial resistance rate during 1994–2016 for *P. aeruginosa*.

Bacteria	Antimicrobial Agent	Susceptibility (%)	Breakpoint	1994	1996	1998	2000	2002	2004	2007	2010	2013	2016	
<i>P. aeruginosa</i> from UTI	LVFX	Susceptible	≤2	41.8	51.5	59.8	62.0	60.0	65.7	72.8	74.1	83.4	91.2	
		Intermediate	4	5.9	8.1	2.7	2.0	4.4	3.6	3.7	4.6	3.8	2.4	
		Resistant	≥8	52.3	40.3	37.4	36.0	35.6	30.7	23.4	21.3	12.9	6.4	
	CPFX	Susceptible	≤1	44.4	57.3	62.6	64.0	62.8	68.0	75.0	76.8	83.7	91.2	
		Intermediate	2	5.6	3.7	1.4	1.3	2.2	2.0	1.7	3.6	2.9	2.6	
		Resistant	≥4	50.0	39.0	36.1	34.7	35.0	29.9	23.3	19.5	13.4	6.2	
	STFX	Susceptible	≤1	–	–	–	–	67.0	71.0	78.6	80.0	89.3	94.2	
		Intermediate	2	–	–	–	–	4.0	6.7	3.4	4.6	3.8	2.6	
		Resistant	≥4	–	–	–	–	29.0	22.3	18.0	15.4	7.0	3.2	
	PIPC	Susceptible	≤16	59.5	67.8	78.1	64.0	77.9	75.0	80.0	83.6	86.8	91.6	
		Intermediate	32–64	9.8	12.9	11.4	17.3	14.1	13.5	12.1	9.7	9.1	6.6	
		Resistant	≥128	30.7	19.3	10.5	18.6	8.0	11.5	8.0	6.7	4.1	1.8	
	CAZ	Susceptible	≤8	70.9	78.6	82.6	81.1	84.5	80.0	86.6	86.0	91.2	92.4	
		Intermediate	16	10.5	9.8	6.4	4.6	4.4	5.4	3.4	3.8	3.8	3.8	
		Resistant	≥32	18.6	11.5	11.0	14.3	11.1	14.6	10.0	10.2	5.0	3.8	
	CPR	Susceptible	≤8	45.1	55.6	67.1	58.9	68.2	67.1	70.5	75.7	79.2	86.2	
		Intermediate	16	14.1	12.2	13.7	12.8	12.5	11.9	13.8	8.4	9.3	7.8	
		Resistant	≥32	40.8	32.2	19.2	28.3	19.3	21.1	15.8	15.9	11.4	6.0	
	MEPM	Susceptible	≤2	–	76.3	74.4	75.3	75.1	74.7	81.2	80.8	86.0	90.2	
		Intermediate	4	–	5.8	5.5	5.1	6.2	7.7	5.1	5.1	5.7	3.2	
		Resistant	≥8	–	18.0	20.1	19.6	18.7	17.6	13.8	14.1	8.2	6.6	
	IPM	Susceptible	≤2	78.4	71.5	77.2	73.7	74.0	74.1	77.2	75.5	83.4	86.3	
		Intermediate	4	6.2	8.1	1.8	3.1	3.4	3.2	2.2	4.1	2.9	2.2	
		Resistant	≥8	15.4	20.3	21.0	23.2	22.7	22.6	20.5	20.4	13.8	11.0	
	AZT	Susceptible	≤8	56.9	62.4	65.3	67.1	69.4	66.3	69.8	72.7	75.7	79.6	
		Intermediate	16	26.5	21.7	14.6	12.5	14.1	12.1	12.4	11.3	11.6	9.6	
		Resistant	≥32	16.7	15.9	20.1	20.4	16.5	21.6	17.8	15.9	12.7	10.8	
	GM	Susceptible	≤4	44.1	50.2	79.0	74.2	82.3	81.2	85.9	89.7	94.8	96.4	
		Intermediate	8	23.2	23.7	7.3	5.9	4.2	5.3	5.1	3.6	2.0	1.0	
		Resistant	≥16	32.7	26.1	13.7	19.9	13.5	13.5	9.0	6.7	3.2	2.6	
	AMK	Susceptible	≤16	82.0	86.4	91.3	87.5	88.9	89.5	92.0	96.4	98.0	98.6	
		Intermediate	32	7.8	3.7	3.7	5.6	5.6	4.0	2.2	1.1	0.7	0.4	
		Resistant	≥64	10.1	9.8	5.0	6.9	5.6	6.6	5.8	2.5	1.3	1.0	
		MDRP	–	4.2	5.1	2.3	6.4	4.2	6.8	4.4	2.3	1.6	0.8	
	<i>P. aeruginosa</i> from RTI	LVFX	Susceptible	≤2	78.9	75.2	85.4	85.2	81.8	81.4	79.2	81.1	88.1	91.7
			Intermediate	4	8.2	9.9	3.1	4.0	7.1	4.8	6.4	6.7	5.2	2.6
			Resistant	≥8	12.9	14.9	11.6	10.8	11.1	13.8	14.4	12.3	6.7	5.7
		CPFX	Susceptible	≤1	84.0	82.9	87.1	86.9	86.0	85.1	82.9	84.2	88.6	90.1
			Intermediate	2	4.1	5.3	2.0	3.1	3.7	3.5	4.6	4.8	4.9	2.6
			Resistant	≥4	11.9	11.8	10.9	10.1	10.3	11.3	12.5	10.9	6.5	7.3
		STFX	Susceptible	≤1	–	–	–	–	88.5	88.3	87.1	87.7	93.7	94.6
			Intermediate	2	–	–	–	–	5.1	6.5	5.8	6.2	3.9	3.8
			Resistant	≥4	–	–	–	–	6.4	5.2	7.1	6.1	2.4	1.6
		PIPC	Susceptible	≤16	76.9	83.9	83.3	79.1	88.9	84.6	80.5	85.2	88.6	86.9
			Intermediate	32–64	7.5	6.5	9.2	11.5	9.3	8.5	13.4	7.6	7.3	9.0
			Resistant	≥128	12.9	9.6	7.5	9.4	1.9	7.0	6.1	7.3	4.1	4.2
		CAZ	Susceptible	≤8	85.7	89.1	89.5	88.0	89.5	86.8	87.8	88.9	92.4	90.3
			Intermediate	16	4.4	3.7	5.8	4.9	3.5	5.1	4.5	4.1	4.1	4.0
Resistant			≥32	9.9	7.1	4.8	7.0	6.9	8.1	7.7	7.0	3.6	5.7	
CPR		Susceptible	≤8	63.3	68.9	75.2	76.5	79.4	80.0	74.6	77.7	81.0	79.4	
		Intermediate	16	18.0	18.0	14.3	12.9	11.0	9.7	11.7	10.6	9.1	9.3	
		Resistant	≥32	18.7	13.0	10.5	10.6	9.6	10.3	13.7	11.7	9.9	11.2	
MEPM		Susceptible	≤2	–	77.0	80.3	74.4	75.0	78.5	76.7	75.2	84.6	87.5	
		Intermediate	4	–	9.3	5.4	9.6	10.5	8.1	8.0	7.1	5.2	4.0	
		Resistant	≥8	–	13.7	14.3	16.0	14.5	13.4	15.3	17.7	10.2	8.5	
IPM		Susceptible	≤2	78.2	69.3	77.2	70.2	65.4	70.8	68.1	69.5	76.8	82.2	
		Intermediate	4	4.1	5.9	1.4	2.3	3.2	3.8	2.5	4.1	2.8	3.5	
		Resistant	≥8	17.7	24.8	21.4	27.5	31.4	25.4	29.4	26.4	20.5	14.4	
AZT		Susceptible	≤8	79.6	77.6	77.9	78.6	82.8	77.7	76.1	73.3	79.2	78.9	
		Intermediate	16	11.2	14.0	12.9	12.4	9.3	11.0	8.8	12.0	9.1	9.0	
		Resistant	≥32	9.2	8.4	9.2	8.9	7.9	11.3	15.2	14.7	11.7	12.1	
GM		Susceptible	≤4	57.5	63.4	92.2	90.1	93.9	92.9	91.2	94.4	97.7	97.1	
		Intermediate	8	31.3	23.3	3.1	4.0	1.9	2.2	4.6	3.3	1.1	1.6	
		Resistant	≥16	11.2	13.4	4.8	5.9	4.2	5.0	4.2	2.3	1.1	1.4	
AMK		Susceptible	≤16	93.9	94.1	99.3	97.4	97.0	97.9	97.3	98.3	99.7	99.0	
		Intermediate	32	4.4	2.8	0.7	0.9	1.2	0.5	0.7	0.6	0.0	0.3	
		Resistant	≥64	1.7	3.1	0.0	1.6	1.9	1.6	1.9	1.1	0.3	0.7	
		MDRP	–	0.3	0.9	0.0	0.5	0.8	1.0	1.4	0.3	0.0	0.5	

LVFX, levofloxacin; CPFX, ciprofloxacin; STFX, sitafloxacin; PIPC, piperacillin; CAZ, ceftazidime; CPR, ceftiprome; MEPM, meropenem; IPM, imipenem; AZT, aztreonam; GM, gentamicin; AMK, amikacin; MDRP, multidrug-resistant *P. aeruginosa*.

Table 7
Percent changes in antimicrobial resistance rate during 1994–2016 for *H. influenzae* and *Acinetobacter* spp.

Bacteria	Antimicrobial Agent	Susceptibility (%)	Breakpoint	1994	1996	1998	2000	2002	2004	2007	2010	2013	2016	
<i>H. influenzae</i>	LVFX	Susceptible	≤2	100.0	100.0	100.0	100.0	99.8	99.9	99.9	99.1	99.8	99.4	
		Resistant	≧4	0.0	0.0	0.0	0.0	0.2	0.1	0.1	0.9	0.2	0.6	
	CPFX	Susceptible	≤1	100.0	100.0	100.0	100.0	99.8	99.9	99.9	99.1	99.7	99.4	
		Resistant	≧2	0.0	0.0	0.0	0.0	0.2	0.1	0.1	0.9	0.3	0.6	
	STFX	Susceptible	≤1	–	–	–	–	100.0	100.0	100.0	100.0	100.0	100.0	
		Resistant	≧2	–	–	–	–	0.0	0.0	0.0	0.0	0.0	0.0	
	ABPC	Susceptible	≤1	81.2	87.0	83.7	76.2	67.0	55.4	42.8	32.1	35.6	32.5	
		Intermediate	2	3.1	1.9	5.8	15.6	21.5	29.9	20.4	23.9	30.8	31.3	
		Resistant	≧4	15.8	11.1	10.5	8.1	11.5	14.7	36.7	43.9	33.5	36.2	
	CVA/AMPC	Susceptible	≤4	100.0	99.0	96.6	93.7	88.7	86.0	67.1	68.9	71.6	–	
		Resistant	≧8	0.0	1.0	3.4	6.3	11.3	14.0	32.9	31.1	28.4	–	
	SBT/ABPC	Susceptible	≤2	–	–	–	–	–	–	–	–	–	–	71.1
		Resistant	≧4	–	–	–	–	–	–	–	–	–	–	28.9
	CCL	Susceptible	≤8	89.4	65.7	76.3	67.0	76.9	55.0	91.1	82.3	80.8	88.2	
		Intermediate	16	5.5	10.5	10.8	11.8	17.2	11.1	5.2	13.8	15.2	8.5	
		Resistant	≧32	5.1	23.8	12.9	21.3	5.9	33.9	3.7	3.9	4.0	3.3	
	CTM	Susceptible	≤1	81.5	63.5	64.7	60.0	51.5	39.4	31.6	22.7	30.0	28.5	
		Resistant	≧2	18.5	36.5	35.3	40.0	48.5	60.6	68.4	77.3	70.0	71.5	
	CFDN	Susceptible	≤1	96.9	87.6	88.5	76.9	55.5	53.1	45.9	39.1	40.6	–	
		Resistant	–	3.1	12.4	11.5	23.1	44.5	46.9	54.1	60.9	59.4	–	
	MINO	Susceptible	≤2	98.6	98.7	99.0	98.0	96.7	99.1	99.6	99.2	98.9	100.0	
		Intermediate	4	1.0	1.0	0.7	1.4	2.9	0.5	0.3	0.2	0.3	0.0	
		Resistant	≧8	0.3	0.3	0.3	0.7	0.5	0.4	0.1	0.6	0.8	0.0	
	CAM	Susceptible	≤8	93.8	95.9	98.0	95.9	100.0	99.0	81.0	85.2	89.0	98.2	
Intermediate		16	4.8	3.8	2.0	4.1	0.0	0.8	17.0	13.2	8.7	0.9		
Resistant		≧32	1.4	0.3	0.0	0.0	0.0	0.2	1.9	1.7	2.3	0.9		
<i>Acinetobacter</i> spp.	LVFX	Susceptible	≤2	–	90.4	95.8	93.1	94.7	92.3	92.5	89.4	91.2	92.2	
		Intermediate	4	–	1.8	2.8	2.0	3.0	2.5	2.3	4.9	4.1	2.5	
		Resistant	≧8	–	7.7	1.4	4.8	2.3	5.2	5.2	5.7	4.7	5.4	
	CPFX	Susceptible	≤1	–	85.6	92.6	90.8	91.4	89.8	88.3	86.8	89.5	88.4	
		Intermediate	2	–	2.2	2.8	1.0	0.4	0.5	1.5	1.0	0.8	1.6	
		Resistant	≧4	–	12.2	4.7	8.2	8.2	9.7	10.2	12.1	9.8	10.0	
	STFX	Susceptible	≤1	–	–	–	–	97.0	95.6	93.8	92.5	93.8	93.8	
		Intermediate	2	–	–	–	–	2.1	2.4	1.8	3.8	3.5	3.6	
		Resistant	≧4	–	–	–	–	0.8	2.0	4.3	3.6	2.7	2.7	
	CTX	Susceptible	≤8	–	–	–	–	–	84.1	46.7	47.5	55.5	55.4	
		Intermediate	16–32	–	–	–	–	–	11.8	45.5	44.7	36.9	38.6	
		Resistant	≧64	–	–	–	–	–	4.2	7.9	7.8	7.6	6.0	
	CAZ	Susceptible	≤8	–	–	–	–	–	93.2	88.0	89.8	89.1	87.9	
		Intermediate	16	–	–	–	–	–	2.2	5.0	2.6	3.3	4.5	
		Resistant	≧32	–	–	–	–	–	4.7	7.0	7.6	7.6	7.6	
	MEPM	Susceptible	≤2	–	–	–	–	–	–	–	–	–	–	96.4
		Intermediate	4	–	–	–	–	–	–	–	–	–	–	0.4
		Resistant	≧8	–	–	–	–	–	–	–	–	–	–	3.1
	IPM	Susceptible	≤2	–	–	–	–	–	97.1	95.3	95.7	96.9	96.7	
		Intermediate	4	–	–	–	–	–	0.4	1.0	1.2	0.4	0.0	
		Resistant	≧8	–	–	–	–	–	2.5	3.7	3.1	2.7	3.3	
	AZT	Susceptible	≤4	–	–	–	–	–	–	–	–	–	–	1.8
		Resistant	≧8	–	–	–	–	–	–	–	–	–	–	98.2
		Intermediate	8	–	–	–	–	–	–	–	–	–	–	–
MINO	Susceptible	≤4	–	98.2	99.5	99.5	98.9	97.6	97.5	96.5	97.1	98.0		
	Intermediate	8	–	1.5	0.0	0.5	0.6	1.9	1.3	1.9	1.2	1.1		
	Resistant	≧16	–	0.4	0.5	0.0	0.4	0.5	1.2	1.6	1.8	0.9		
GM	Susceptible	≤4	–	–	–	–	–	–	–	–	–	–	92.6	
	Intermediate	8	–	–	–	–	–	–	–	–	–	–	2.1	
	Resistant	≧16	–	–	–	–	–	–	–	–	–	–	5.3	
AMK	Susceptible	≤16	–	–	–	–	–	–	–	–	–	–	98.0	
	Intermediate	32	–	–	–	–	–	–	–	–	–	–	0.6	
	Resistant	≧64	–	–	–	–	–	–	–	–	–	–	1.4	

LVFX, levofloxacin; CPFX, ciprofloxacin; STFX, sitafloxacin; ABPC, ampicillin; CVA/AMPC, clavulanic acid/amoxicillin; SBT/ABPC, sulbactam/ampicillin; CCL, cefaclor; CTM, cefotiam; CFDN, cefdinir; MINO, minocycline; CAM, clarithromycin; CTX, cefotaxime; CAZ, ceftazidime; MEPM, meropenem; IPM, imipenem; AZT, aztreonam; GM, gentamicin; AMK, amikacin.

H. influenzae) in 2004, 41.7% (3.0%) in 2007, 34.8% (3.5%) in 2010, 35.6% (2.6%) in 2013, and 36.9% (4.4%) in 2016.

3.6. *Acinetobacter* spp.

The susceptibility rate to FQs was almost constant around 90% during the study period (Table 7). MEPM, IPM, MINO, GM, and AMK also maintained high activity with a susceptibility rate of over

92.2% in 2016. One isolate of multidrug-resistant *Acinetobacter* spp. (MDRA, CPFX: ≥4 µg/mL, IPM: ≥16 µg/mL and AMK: ≥32 µg/mL) was detected in 2013, but no MDRA was observed in 2016. Because AMK was not tested before 2010, both CPFX and IPM-resistant strains were investigated. The resistant strains to CPFX and IPM were isolated at 0.5% (4 strains) in 2004, 1.7% (10 strains) in 2007, 1.7% (10 strains) in 2010, 1.8% (9 strains) in 2013, and 2.0% (9 strains) in 2016.

4. Discussion

Antimicrobial susceptibility testing was conducted continuously from 1994 to 2016. We have already reported the susceptibility trends from 1994 to 2002 [9]. In that paper, it was reported that the resistance rate to FQs against MRSA was higher than that against MSSA, and we discussed that FQ-resistance may be linked to the presence of *mec* locus. It is considered that the same trend will continue in 2016. However, the resistance rate to IPM markedly decreased because there has been an overall decline in use of carbapenems. DAP is a novel cyclic lipopeptide antibiotic against Gram-positive bacteria and was approved in 2011 in Japan, and DAP-resistant isolates were reported in USA, Europe, Taiwan and the other areas around the world [17]. In the study, one DAP-resistant MRSA, which was also resistant to FQs, β -lactams, and macrolides but susceptible to VCM and linezolid, was found in the Chubu-area of Japan in 2016. This is one of the future concerns and an important point for surveillance. CA-MRSA became a major concern worldwide in the early 2000s, thus we estimated the isolation frequency according to the susceptibility patterns to LVFX, MINO, and CAM. In Japan, the frequency of CA-MRSA is considered lower than in USA and Europe, where the major clone is USA300 [18], however, Nakamura et al. reported 4 cases of CA-MRSA infection [19], and Yamaguchi et al. reported an increase in the USA300 clone among patients suffering skin and soft tissue infections in Japan [20]. As such, continued surveillance is important.

The common respiratory pathogens of *S. pneumoniae*, *S. pyogenes*, and *H. influenzae* continue to show a high susceptibility to FQs, especially to STFX. There are three main resistance mechanisms to FQs; i) mutation in type II topoisomerases reducing binding affinity, ii) overexpression of efflux pumps facilitating export, and iii) plasmid-mediated *qnr* genes encoding proteins thought to sterically prevent FQ to interact with topoisomerases [21]. We investigated the amino acid substitution of major sites in QRDR of target enzymes, and major mutations were found in all LVFX-resistant *S. pneumoniae*, *S. pyogenes*, and *H. influenzae* tested except for seven *S. pneumoniae* (LVFX MIC: 8, 16 and 64 $\mu\text{g}/\text{mL}$, isolated in 2013 and 2016). The strain with LVFX MIC of 64 $\mu\text{g}/\text{mL}$ was also resistant to ABPC, CTRX, and MINO, and intermediate to STFX, IPM and CAM. There are reports in Japan and the United Kingdom that, after the introduction of pneumococcal conjugate vaccines, multidrug-resistant serotype 15A increased [22,23], thus, there are concerns about multidrug-resistant *S. pneumoniae*. QRDR alteration was found in 30.8% (61/198 strains) and 87.5% (21/24 strains) of LVFX-susceptible strains with LVFX MIC of 0.5–2 $\mu\text{g}/\text{mL}$ in *S. pyogenes* and *H. influenzae*, respectively. These susceptible strains may become resistant if one more mutation is acquired. Regarding *S. pyogenes*, an overall decreasing trend in macrolide-resistance has been reported recently, mostly in Europe, but an increasing trend was reported in Japan until 2012 [24]. In our study, CAM showed a recovery of its susceptibility after 2010.

Honda et al. reported that 60% (62/102 strains) of *H. influenzae* isolated in 2016–2018 were BLNAR, 92% (57/62 strains) of BLNAR was high-level ABPC-resistant, and 4/62 BLNAR strains showed decreased FQ susceptibility, which possessed amino acid substitution in GyrA [25]. BLPACR has emerged after 2007 among young children in Japan [26]. In this study, no increase of BLNAR was observed after 2010, but an increase of BLPACR was suggested. This is also an important point for continued surveillance.

The LVFX-resistant rate showed a continuous increase in *E. coli* although the upward trend was slowing down, however, another Enterobacteriaceae member, *K. pneumoniae*, showed a low resistance rate to FQs. In *E. coli*, amino acid substitution(s) in QRDR was observed not only in LVFX resistant strains but also in many LVFX

susceptible strains (LVFX MIC = 0.06–2 $\mu\text{g}/\text{mL}$), and some strains with LVFX MIC of 0.5–2 $\mu\text{g}/\text{mL}$ had double alterations in GyrA and ParC as well as LVFX resistant strains. On the other hand, in *K. pneumoniae*, there was amino acid substitution(s) in the LVFX resistant strains while the lower rate compared in *E. coli*, and in the LVFX susceptible strains only a GyrA alteration was observed in just a small number of strains. It is highly likely that the accumulation of such target enzyme mutations has influenced the progress of LVFX resistance, but the reason for the difference in accumulation rate between *E. coli* and *K. pneumoniae* is not clear. However, the slowing of upward trend seems to suggest that the appropriate use of antibiotics is proceeding. Shams et al. reported that ESBL production was found in 58.3% and 69.8% of FQ-resistant *E. coli* and *K. pneumoniae*, respectively, and class 1 and 2 integrons were found in 73.5% of MDR isolates [27]. Awareness of the risk of emergence of MDR *E. coli* should be raised. CRE is also a global problem [6], however the frequency of IPM-resistant *E. coli* and *K. pneumoniae* was very low (3 isolates during the study period) in the study and the susceptibility of IPM increased gradually from 2007 to 2016.

Regarding *P. aeruginosa*, it was summarized as having a susceptibility to FQs of over 90% in both UTI and RTI strains in 2016 and a continuous decrease in FQ-resistant *P. aeruginosa* was noted, especially among isolates from UTI. Also, the isolation frequency of multidrug-resistant *P. aeruginosa* decreased. This suggests that appropriate antimicrobial therapy may be progressing. The frequency of extensively drug-resistant *Acinetobacter baumannii* significantly increased from 11.1% in 2004 to 60.4% in 2014 in China [28], and the global spread of multidrug-resistant *A. baumannii* has been reported [7]. Although multidrug-resistant *Acinetobacter* spp. was very rare in Japan, outbreaks were reported sporadically [29].

Outcome indices for the national action plan called for a resistance rate to PCG in *S. pneumoniae* of under 15%, an isolation frequency of MRSA among *S. aureus* of under 20%, a resistance rate to FQ in *E. coli* of under 25%, a resistance rate to carbapenem in *P. aeruginosa* of under 10%, and a resistance rate to carbapenem in *E. coli* and *K. pneumoniae* of under 0.2% by 2020. In this surveillance, the latest resistance rate to PCG in *S. pneumoniae* was 6.4%, and the resistance rate to IPM in *E. coli* and *Klebsiella* spp. was 0.1% and 0.2%, respectively, thus the outcome indices have already been achieved. However, the resistance rate to LVFX in *E. coli* was 34.2% and the resistance rate to IPM in *P. aeruginosa* from UTI and RTI was 11.0% and 14.4%, respectively, which have not achieved the outcome indices. The isolation frequency of MRSA among *S. aureus* was not calculated because the clinical isolates were corrected as MSSA and MRSA.

Among the six goals stipulated in the AMR action plan in Japan, one is continuous monitoring of antimicrobial resistance and usage of antibacterials, and appropriate understanding of the signs of change and spread of antimicrobial resistance. The trend over 20 years in the study showed some important points for future surveillance. Recently, nationwide surveillance has been conducted from 2006 by the Japanese surveillance committee and the susceptibility pattern has been reported [30–33]. The current state of emergence of multidrug resistance in the world and the delay of drug development, appropriate usage of existing antimicrobial agents and continuous surveillance are important issues.

Conflicts of interest

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