***In-vitro* activity of levofloxacin against bacterial pathogens collected in China A ten-year (2009-2018) retrospective study**

LU GAN

Peking University First Hospital

Yun Li (✉ liyun19702@sina.com)

Yuan Lv

Peking University First Hospital

Bo Zheng

Peking University First Hospital

Research

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Abstract

Background This study was designed to evaluate the *in-vitro* activity of levofloxacin against bacterial pathogens collected from Chinese hospitalized patients between 2009 and 2018, and analysis the trends of levofloxacin resistance in China.

Methods In this analysis, antimicrobial minimum inhibitory concentrations (MICs) experiments with levofloxacin and controls against a range of Gram-positive and Gram-negative bacteria collected from 2009 to 2018. MICs were determined using the agar dilution method according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) [2019]. Antimicrobial susceptibility was determined using CLSI breakpoints. Statistical tests were analysed by Statistical Package for the Social Sciences software (SPSS, Inc., Chicago, IL, USA), calculating the MIC₉₀.

Results In the past decade, the antibacterial activities of levofloxacin against *Escherichia coli*, *Enterobacter cloacae*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*, which were common gram-negative bacteria, were stable; the resistance rates of these bacteria had no significant changes or decreased slightly. The levofloxacin resistance rates of *Staphylococcus aureus* and *Enterococcus faecalis*, which were gram positive bacteria, decreased from 48.4% and 36.9% in 2009-2010 to 22.8% and 25.7% in 2017-2018, respectively, respectively. The levofloxacin resistance rate among *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Streptococcus pneumoniae*, the common pathogens of community acquired pneumonia (CAP), were less than 3%. The levofloxacin resistance rates for *Klebsiella pneumoniae* and *Acinetobacter baumannii* increased. No difference in the levofloxacin resistance rates by age group (18-64, 65-74, ≥75 years old age groups) was observed. The resistance rate of strains isolated from ICU patients was usually 10-20% higher than that of non-ICU patients.

Conclusion In recent ten years, levofloxacin has continued to be active *in-vitro* against the strains in its antibacterial spectrum. No significant change of resistance rates was observed and it still has a good antibacterial effect on the main pathogenic bacteria of community-acquired pneumonia, such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Klebsiella pneumoniae* and MSSA.

Background

Levofloxacin is a fluoroquinolone antibiotic and is the optical S-(−) isomer of the racemic drug substance ofloxacin [1]. It has the same spectrum with ofloxacin but is at least twice as active as ofloxacin. Levofloxacin exert its antibacterial effects by inhibiting the activity of DNA gyrase and stopping bacterial DNA synthesis and duplication. It interferes with critical processes in the bacterial cell, such as DNA replication, transcription, repair and recombination, by inhibiting type II topoisomerases. Human cells lack these topoisomerases, but they are essential for bacterial DNA replication, thereby allowing these agents to be specific for bacterial cells [2]. It is a broad-spectrum antibacterial agent with high activity against *Haemophilus influenzae*, *Legionella pneumophila*, *Neisseria gonorrhoeae*, most of *Enterobacteriaceae* and other gram-negative bacteria; it also has antibacterial effect on *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*, Gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pneumoniae*, but it has poor efficacy on anaerobic bacteria and *Enterococcus*. Oral absorption of levofloxacin is rapid and essentially complete, with high concentrations in plasma and a relatively long plasma half-life; 80%–86% of this drug is excreted in the urine, which related to kidney function. It is mainly used for the infection of bacterial pathogens in respiratory system, urogenital system, digestive system, bone and joints, skin structure and soft tissue, and it is also used in combination with isoniazid and rifampicin to treat human tuberculosis [3]. Levofloxacin is well tolerated and induces minimal adverse reactions, it occasionally leads to shock, hypersensitivity reaction, gastrointestinal reaction and increase of blood urea nitrogen (BUN); articular cartilage disease have been associated with levofloxacin use, so we advise that individuals under 18 years old and pregnant women to avoid the use of it [4]. The Institute of clinical pharmacology of Peking University has carried out China antimicrobial resistance surveillance since 1998. Now, the surveillance results of levofloxacin in 2009–2018 are summarized as follows.

Methods

Bacterial isolates

The China Antimicrobial Resistance Surveillance Trial (CARST) programme was established to monitor the applications of antibiotics and the trend in antimicrobial resistance among clinical isolates, providing scientific data for the treatment of clinical infection. All isolates were collected biennially by the CARST programme over five consecutive 2 year periods and were sent to a central laboratory (Institute of Clinical Pharmacology, Peking University First Hospital). One isolate per species per patient was collected to avoid repetitive counts. Every strain to be tested was recovered and purified before the experiment to ensure viability and purity of the bacteria. All isolates were collected from clinical samples provided by hospitalized patients during routine examination without harming patients' interests.

Antimicrobial Susceptibility Testing

MICs were determined by the standard two-fold agar dilution method and interpretative breakpoint criteria were in accordance with those recommended by the CLSI [2019] [5] guidelines, See the notes after the tables about the agents that were not critical (refer to European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2019) [6], or similar drugs, or were not calculated.

Data Collection And Statistical Analysis

We collected and recalculated the antimicrobial minimum inhibitory concentrations (MICs) of levofloxacin and control drugs against clinical bacterial isolates obtained as part of the CARST programme over ten consecutive 2 year periods between January 2009 and June 2018 (2009–10; 2011–12; 2013–14; 2015–

16;2017–18). The susceptibility rate and resistance rate of isolates to antimicrobial agents were determined by using CLSI2019 guidelines. If the number of strains is less than 20, no statistical calculation will be made. Statistical tests were analysed by Statistical Package for the Social Sciences software (SPSS, Inc., Chicago, IL, USA), calculating the MIC₉₀.

Results

Enterobacteriaceae

The MIC₉₀, susceptibility rate and resistance rate of levofloxacin and other antibiotics against *Escherichia coli* are shown in Table 1.

Table 1
Antimicrobial activity of antimicrobial agents against *E.coli* by year

Antibiotic	2009–2010(n = 1156)			2011–2012(n = 694)			2013–2014(n = 806)			2015–2016(n = 721)			2017–2018(n = 738)		
	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R
Levofloxacin	32	31.3	66.4	32	32.0	67.3	32	32.3	64.0	32	37.3	59.8	32	34.6	59.5
ciprofloxacin	128	27.3	69.0	128	30.1	68.0	128	31.1	66.4	128	34.1	61.9	128	33.5	61.9
P/T	8	93.5	4.4	8	90.6	6.9	32	89.8	5.5	16	92.1	5.0	16	90.7	6.2
Cefazolin	> 256	21.3	75.3	> 256	22.2	74.6	> 256	16.0	76.7	> 256	22.5	71.2	> 256	27.0	65.7
Cefoxitin	NT	NT	NT	NT	NT	NT	64	73.6	17.9	32	74.1	14.3	32	73.8	16.1
Cefotaxime	> 256	27.6	71.9	256	28.0	71.6	> 256	29.4	70.2	> 256	35.0	64.3	> 256	40.0	59.5
Ceftriaxone	> 256	27.4	72.2	256	27.5	72.3	> 256	29.7	70.1	> 256	35.4	64.3	> 256	40.1	59.6
Ceftazidime	64	62.4	33.4	64	57.3	35.0	128	55.7	38.5	128	61.9	31.5	64	59.5	33.1
CSL	64	68.4	13.5	32	80.4	9.1	64	71.2	11.4	64	73.5	11.0	64	79.0	10.3
Cefepime	64	39.6	34.9	32	42.9	26.5	128	41.2	38.6	64	45.8	33.8	64	52.3	28.3
Imipenem	0.12	99.6	0.3	0.12	99.3	0.4	0.25	98.6	1.2	0.25	97.5	2.4	0.12	98.1	1.8
Meropenem	0.03	99.4	0.4	0.03	99.1	0.4	0.06	98.4	1.5	0.03	98.1	1.8	0.03	98.1	1.6
gentamicin	128	40.7	58.5	128	40.8	57.6	128	48.8	50.7	128	53.1	46.6	256	53.1	46.3
Amikacin	8	92.7	6.4	4	93.9	5.8	4	96.9	2.9	8	96.8	2.8	8	95.9	2.8
S, Susceptible rate (%); R, Resistant rate (%); P / T, piperacillin / tazobactam; CSL, Cefoperazone / sulbactam; Cefoperazone / sulbactam were determined according to the standard of cefoperazone (susceptibility: ≤ 16 mg / L; resistance ≥ 64 mg / L); NT, not detected.															

The MIC₉₀ of levofloxacin to *Escherichia coli* was unchanged in the past decade, and the resistance rate appeared stable with slightly decrease, ranged from 67.3–59.5%. The resistance rate, trend of levofloxacin and the cumulative inhibitory percentage of MIC were similar to those of the third generation cephalosporins such as cefotaxime and ceftriaxone (Table 1) (Fig. 1). To investigate the effect of ESBLs on the resistance of *E.coli*, we compared the resistance rate of levofloxacin of ESBLs producing and no-producing strains. The ESBL-positive strains showed resistance rates to levofloxacin over 70%, about 30 percentage points higher than that of ESBL-negative strains, while the resistance rate of strains isolated from ICU to levofloxacin is not significantly higher than that of non-ICU strains (by 13 percentage points in 2017–2018 than that of last period). The results of strains isolated from the different sources show that the drug resistance rate of strains from urine source is slightly higher than that of strains from blood and sputum source. The high resistance rate of strains from sputum source in 2017–2018 may be related to the small number of strains (19 strains). Comparison of different age group shows no difference in the levofloxacin resistance rates by age group (18–64, 65–74, ≥ 75 year old age groups) (Fig. 2).

In recent ten years, there was an increase in the MIC₉₀ for levofloxacin against *Klebsiella pneumoniae*. Resistance rate of levofloxacin against *Klebsiella pneumoniae* has increased from 24.9% in 2009–2010 to 36.5% in 2017–2018 and the resistance rate of ciprofloxacin increased from 29.3% in 2009–2010 to 45.0% in 2017–2018, with the most significant increase in 2017–2018 both, which are about 8% higher than the previous period. The piperacillin / tazobactam resistance rate increased from 10.7% in 2009–2010 to 25.6% in 2017–2018 whereas aminoglycosides resistance rate was stable; the resistance rate of carbapenems increased significantly from about 1.0% in 2009–2010 to about 19.0% in 2017–2018, and different from fluoroquinolones, it increased at a constant rate (Table 2). Figure 3 shows that the bacteriostasis cumulative percentage curve of levofloxacin in 2017–2018 slightly deviates to the right which means high MIC. From 2009 to 2016, the resistance rate of ESBL-producing *Klebsiella pneumoniae* to levofloxacin increased, while that of non-ESBL-producing *Klebsiella pneumoniae* was significantly lower, stable at 15.7% – 17.9%; the resistance rate of ESBL-producing *Klebsiella pneumoniae* to levofloxacin in 2017–2018 fell to the level of 2013–2014, while the resistance rate of non-ESBL-producing *Klebsiella pneumoniae* increased to 30.9%; the resistance rate of carbapenems also showed the same trend (the results were not shown). Compared with non-ICU, the resistance rate of strains isolated from ICU was higher and increased faster. There was no significant difference in levofloxacin resistance between the strains isolated from blood and sputum. The resistance rate to levofloxacin increased with the increase of age in 2009–2012, but no difference in the levofloxacin resistance rates between close age groups was observed (Table 4).

Table 2
Antimicrobial activity of antimicrobial agents against *Klebsiella pneumoniae* by year

Antibiotic	2009–2010(n = 845)			2011–2012(n = 510)			2013–2014(n = 584)			2015–2016(n = 563)			2017–2018(n = 602)		
	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R
Levofloxacin	16	68.6	24.9	32	64.1	29.2	16	61.3	29.8	32	63.4	29.0	64	52.7	36.5
ciprofloxacin	32	59.2	29.3	64	55.5	34.1	64	51.4	38.7	64	56.1	36.6	128	47.8	45.0
P/T	128	86.0	10.7	128	86.5	11.2	> 256	83.9	13.5	> 256	76.2	21.0	> 256	71.9	25.6
Cefazolin	> 256	41.4	57.0	> 256	42.5	55.7	> 256	40.4	53.1	> 256	45.8	47.4	> 256	40.2	53.0
Cefoxitin	NT	NT	NT	NT	NT	NT	256	74.3	22.3	256	73.4	22.4	> 256	66.8	29.4
Cefotaxime	256	47.1	51.5	256	47.8	50.4	> 256	50.5	49.0	> 256	56.3	43.2	> 256	51.5	48.2
Ceftriaxone	256	47.5	51.5	256	48.2	50.8	> 256	50.7	48.6	> 256	56.5	43.0	> 256	52.0	48.0
Ceftazidime	128	67.6	26.6	128	65.5	28.8	256	64.7	32.2	> 256	66.1	29.8	> 256	61.8	34.7
CSL	64	75.3	13.1	64	74.3	12.5	128	69.3	20.2	> 256	70.5	22.6	> 256	65.0	28.7
Cefepime	32	64.4	22.2	32	62.4	23.5	128	59.6	30.5	128	63.1	27.0	256	57.1	35.0
Imipenem	0.12	98.7	1.2	0.25	96.1	3.5	2	89.6	9.1	32	84.9	14.6	64	80.1	19.1
Meropenem	0.03	98.6	0.8	0.06	95.7	4.1	4	89.4	10.1	16	84.9	14.4	128	79.9	19.6
gentamicin	> 256	60.9	38.1	256	62.7	36.3	128	68.2	31.2	256	68.9	30.2	> 256	69.8	29.4
Amikacin	> 256	88.3	11.2	8	90.4	9.4	8	91.8	8.2	> 256	87.2	11.7	> 256	86.7	13.1
S, Susceptible rate (%); R, Resistant rate (%); P / T, piperacillin / tazobactam; CSL, Cefoperazone / sulbactam; Cefoperazone / sulbactam were determined according to the standard of cefoperazone (susceptibility: ≤ 16 mg / L; resistance ≥ 64 mg / L); NT, not detected.															

Table 4
Antimicrobial activity of antimicrobial agents against *Acinetobacter baumannii* by year

Antibiotic	2009–2010(n = 794)			2011–2012(n = 600)			2013–2014(n = 647)			2015–2016(n = 626)			2017–2018(n = 671)		
	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R
Levofloxacin	16	34.3	40.9	16	25.8	54.3	16	25.8	63.5	32	25.1	62.9	32	29.1	65.1
ciprofloxacin	128	28.1	71.2	128	23.8	76.0	128	24.7	75.3	128	24.6	75.1	256	28.8	71.2
P/T	> 256	27.3	63.0	> 256	24.2	71.5	> 256	24.3	74.7	> 256	12.8	76.0	> 256	26.1	72.6
Cefotaxime	> 256	21.2	71.5	> 256	12.8	74.5	> 256	12.7	75.0	> 256	10.1	74.8	> 256	13.9	71.4
Ceftriaxone	> 256	14.6	71.9	> 256	9.7	74.8	> 256	4.9	74.3	> 256	6.2	75.4	> 256	8.5	71.5
Ceftazidime	> 256	28.6	70.8	> 256	25.7	73.7	> 256	25.5	73.9	> 256	25.6	73.2	> 256	26.7	71.5
CSL	64	—	—	128	—	—	128	—	—	256	—	—	128	—	—
Cefepime	128	28.2	56.2	256	26.0	62.3	256	24.0	72.5	256	24.1	71.9	256	26.8	70.6
Imipenem	64	42.2	55.8	64	33.2	65.3	64	28.6	70.5	64	29.1	69.8	64	27.9	71.4
Meropenem	64	40.8	57.1	128	31.5	67.3	128	28.3	70.6	64	27.3	71.4	64	27.7	71.7
gentamicin	> 256	25.3	73.7	> 256	24.7	75.0	> 256	26.7	72.6	> 256	26.4	72.0	> 256	30.3	65.4
Amikacin	> 256	32.0	67.1	> 256	33.2	66.2	> 256	33.7	66.0	> 256	34.0	65.5	> 256	40.5	58.9
S, Susceptible rate (%); R, Resistant rate (%); P / T, piperacillin / tazobactam; CSL, Cefoperazone / sulbactam; —, no criteria.															

The resistance rate of *Enterobacter cloacae* to levofloxacin has declined over the past decade, from 2009–2010(29.5%) to 2017–2018(18.3%). In addition to carbapenem resistance rate increased, other test drug resistance rate also showed a downward trend (Table 3).

Table 3
Antimicrobial activity of antimicrobial agents against *Enterobacter cloacae* by year

Antibiotic	2009–2010(n = 241)			2011–2012(n = 183)			2013–2014(n = 207)			2015–2016(n = 202)			2017–2018(n = 186)		
	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R
Levofloxacin	16	67.2	29.5	16	66.1	24.6	16	70.5	21.7	32	71.3	20.3	8	72.0	18.3
ciprofloxacin	32	63.1	33.2	16	62.8	31.1	32	65.7	25.6	32	69.3	22.3	16	69.4	21.5
P/T	> 256	72.6	19.9	128	77.0	14.2	128	78.7	13.0	> 256	69.3	22.8	128	78.0	14.0
Cefotaxime	> 256	44.0	53.9	> 256	43.7	55.7	> 256	54.6	42.5	> 256	50.5	46.5	256	52.7	44.6
Ceftriaxone	> 256	44.0	54.4	256	43.2	55.7	> 256	54.1	43.5	> 256	51.5	45.0	> 256	54.8	43.0
Ceftazidime	256	53.9	43.6	256	49.2	44.8	128	61.8	34.3	256	58.9	40.1	256	63.4	33.3
CSL	128	58.5	37.3	64	53.0	37.7	128	61.8	31.9	128	59.9	37.1	64	78.5	13.4
Cefepime	64	67.2	20.7	64	66.7	19.1	32	78.3	16.4	32	71.3	16.3	32	77.4	14.5
Imipenem	0.25	98.3	0.8	0.25	95.6	3.3	1	92.8	4.8	4	88.6	10.4	1	90.9	7.5
Meropenem	0.12	97.9	1.2	0.12	95.6	3.3	0.12	94.2	3.9	1	90.6	9.4	2	89.8	8.1
gentamicin	256	60.6	36.9	128	71.0	28.4	128	78.3	18.4	128	81.7	15.8	128	87.1	12.4
Amikacin	16	90.9	9.1	16	90.2	9.8	8	96.6	2.9	> 256	8.0	91.0	8	96.2	3.2
S, Susceptible rate (%); R, Resistant rate (%); P / T, piperacillin / tazobactam; CSL, Cefoperazone / sulbactam; Cefoperazone / sulbactam were determined according to the standard of cefoperazone (susceptibility: ≤ 16 mg / L; resistance ≥ 64 mg / L).															

Other Gram-negative Organisms

From 2009 to 2014, the resistance rate of *Acinetobacter baumannii* to levofloxacin increased from 40.9–63.5%, and then remained stable at about 65%. The same trend was observed in other test drugs, carbapenems increased from about 55% to about 70% (Table 4), and Fig. 5 also showed the bacteriostasis cumulative percentage curve shift to the right. The levofloxacin resistance rate of imipenem sensitive strains is less than 20%, significantly lower than that of imipenem insensitive strains. The resistance rate of strains isolated from ICU is consistently about 20 percentage points higher than that of non-ICU strains. There was no significant difference in resistance rate between the strains from blood and sputum and among age groups (Fig. 6).

In recent ten years, there is almost no change in the MIC₉₀ value and resistance rate of *Pseudomonas aeruginosa* against levofloxacin, and the resistance rate remained stable at about 30% (Table 5). Figure 7 shows that the proportion of strains with MIC value lower than 0.25 mg/l of levofloxacin decreased. The levofloxacin resistance rate of imipenem sensitive strains was significantly lower than that of non-sensitive strains. The resistance rate of strains isolated from ICU was most > 10 percentage points higher than that of non-ICU strains. The resistance rate of strains isolated from sputum was higher than that of strains isolated from blood, but the difference changes with the year, and there was no significant difference in resistance rate among age groups (Fig. 8).

Table 5
Antimicrobial activity of antimicrobial agents against *Pseudomonas aeruginosa* by year

Antibiotic	2009–2010(n = 665)			2011–2012(n = 620)			2013–2014(n = 644)			2015–2016(n = 658)			2017–2018(n = 680)		
	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R
Levofloxacin	32	59.8	30.7	16	57.6	34.6	16	61.5	28.3	16	59.0	30.2	16	62.8	29.0
ciprofloxacin	16	64.5	28.1	16	66.5	28.4	8	71.4	21.7	4	69.1	23.1	8	71.0	21.6
P/T	128	71.1	17.3	128	69.0	15.0	256	69.1	19.6	256	70.1	18.5	256	76.6	15.9
Ceftazidime	128	75.5	19.8	64	75.8	18.7	64	77.0	17.1	64	76.7	18.5	64	80.9	15.0
CSL	128	—	—	128	—	—	128	—	—	128	—	—	128	—	—
Cefepime	64	74.1	14.7	32	76.5	12.1	32	76.6	13.2	32	77.2	12.8	32	81.3	10.9
Imipenem	16	63.2	33.7	16	65.2	29.0	32	67.1	28.0	32	64.6	29.5	32	67.5	29.7
Meropenem	16	64.8	28.0	16	66.1	24.8	16	67.7	24.1	16	67.6	26.6	16	68.5	23.2
Gentamicin	> 256	65.3	33.1	> 256	71.8	25.3	> 256	78.9	18.3	256	83.0	13.5	16	85.7	10.6
Amikacin	> 256	78.9	20.0	> 256	84.4	14.5	32	89.4	9.3	16	92.6	6.8	8	94.7	4.3
S, Susceptible rate (%); R, Resistant rate (%); P / T, piperacillin / tazobactam; CSL, Cefoperazone / sulbactam; —, no criteria.															

The levofloxacin resistance rate of *Stenotrophomonas maltophilia* was below 10% from 2009 to 2016, and increased slightly from 2017 to 2018. The resistance rates of levofloxacin, ticarcillin / clavulanate and minocycline were lower than 20%, while the intermediate rate of ticarcillin / clavulanate was nearly 50% (Table 6). Levofloxacin has a good antibacterial effect on *Haemophilus influenzae*, and the insensitivity rate is basically below 1% (Table 7).

Table 6
Antimicrobial activity of antimicrobial agents against *Stenotrophomonas maltophilia* by year

Antibiotic	2009–2010(n = 245)			2011–2012(n = 148)			2013–2014(n = 176)			2015–2016(n = 166)			2017–2018(n = 167)		
	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R
Levofloxacin	4	89.8	9.4	4	85.1	8.8	4	86.9	9.7	8	86.7	10.2	8	82.6	15.0
Ticarcillin / clavulanic acid	64	69.4	9.4	64	50.7	9.5	128	47.2	17.6	128	48.8	14.5	128	38.9	14.4
Ceftazidime	128	47.7	44.5	256	30.4	62.2	256	40.9	51.1	256	34.3	54.2	256	33.5	58.1
minocycline	1	98.4	0.0	4	95.3	2.7	2	94.9	2.8	2	98.8	1.2	4	95.2	3.0
SMZ-TMP	0.5	91.4	8.6	64	83.1	16.9	1	93.8	6.2	4	88.0	12.0	8	59.9	40.1
chloramphenicol	32	75.6	12.2	64	43.2	31.8	32	48.3	23.3	32	61.4	15.7	64	36.5	28.1
S, Susceptible rate (%); R, Resistant rate (%); SMZ-TMP: compound sulfamethoxazole															

Table 7
Antimicrobial activity of antimicrobial agents against *Haemophilus influenzae* by year

Antibiotic	2009–2010(n = 88)			2011–2012(n = 184)			2013–2014(n = 142)			2015–2016(n = 210)			2017–2018(n = 252)		
	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R
Levofloxacin	0.5	100.0	—	0.25	98.9	1.1*	0.12	100.0	—	0.5	99.5	0.5*	0.5	99.6	0.4*
ciprofloxacin	0.5	97.7	2.3*	0.25	98.4	1.6*	0.25	98.6	1.4*	0.5	98.1	1.9*	1	96.8	3.2*
Moxifloxacin	0.5	96.6	3.4*	0.25	97.8	2.1*	0.5	96.5	3.5*	0.5	96.2	3.8*	1	94.8	5.2*
ampicillin	64	65.9	21.6	256	48.9	33.7	32	59.2	28.9	256	40.5	43.3	>256	27.4	63.1
P/T	0.12	100.0	0.0	0.25	99.5	0.5	0.12	100.0	0.0	0.12	100.0	0.0	0.25	100.0	0.0
Cefotaxime	0.06	100.0	—	0.06	98.4	1.6*	0.12	100.0	—	1	97.6	2.4*	1	96.4	3.6*
Ceftriaxone	0.016	100.0	—	0.016	100.0	—	0.03	100.0	—	0.25	100.0	—	0.25	100.0	—
Ceftazidime	0.25	100.0	—	0.25	100.0	—	0.25	100.0	—	0.5	98.6	1.4*	1	98.8	1.2*
Cefepime	0.25	98.9	1.1*	0.5	98.4	1.6*	1	97.9	2.1*	2	94.8	5.2*	2	90.9	9.1*
Imipenem	8	80.7	19.3*	16	60.9	39.1*	16	76.8	23.2*	32	50.0	50.0*	16	61.9	38.1*
Meropenem	0.5	100.0	—	0.5	96.7	3.3*	0.5	90.8	9.2*	0.5	92.9	7.1*	0.5	94.8	5.2*
Azithromycin	2	93.2	6.8*	4	92.9	7.1*	4	90.8	9.2*	128	76.7	23.3*	64	74.2	25.8*
S, Susceptible rate (%); R, Resistant rate (%); *insensitivity rate; —, no criteria.															

Gram-positive Organisms

In the past ten years, the resistance rate of *Staphylococcus aureus* to levofloxacin and other fluoroquinolones has decreased continually (Table 8, Fig. 9). This trend also existed in β -lactams and aminoglycosides, while the resistance rate of macrolides changed little. The resistance rate of MRSA was significantly higher than that of MSSA, and the resistance rate of strains isolated from ICU was 10–20 percentage points higher than that of non-ICU strains. The resistance rate of strains from sputum was higher than that of strains from blood from 2009 to 2016, but that of strains from blood was slightly higher in 2017–2018. The resistance rate of strains isolated from the elderly aged 75 and over have the highest resistance rate to levofloxacin, and there is no significant difference between adults and young elderly group (Fig. 10).

Table 8
Antimicrobial activity of antimicrobial agents against *Staphylococcus aureus* by year

Antibiotic	2009–2010(n = 630)				2011–2012(n = 687)				2013–2014(n = 751)				2015–2016(n = 717)				2017–2018(n = 717)	
	MIC ₉₀	S	I	R	MIC ₉₀	S	I	R	MIC ₉₀	S	I	R	MIC ₉₀	S	I	R	MIC ₉₀	S
Levofloxacin	32	50.8	0.8	48.4	32	56.9	0.6	42.5	64	70.0	0.7	29.3	32	73.2	0.3	26.5	32	76.1
ciprofloxacin	64	45.9	4.4	49.7	128	52.1	4.8	43.1	128	62.3	5.3	32.4	64	64.4	7.8	27.8	64	66.4
Moxifloxacin	8	51.1	2.5	46.3	8	57.8	3.6	38.6	8	70.3	0.5	29.3	8	73.5	0.3	26.2	8	77.1
Cefoxitin	256	48.4	—	51.6	256	55.5	—	44.5	> 256	56.5	—	43.5	256	60.7	—	39.3	256	67.6
Gentamicin	128	47.9	1.9	50.2	64	56.5	4.4	39.2	128	63.8	2.3	34.0	64	69.9	2.5	27.6	64	75.4
Azithromycin	> 256	31.1	1.9	67.0	> 256	23.6	1.7	74.7	> 256	29.2	0.8	70.0	> 256	34.3	0.4	65.3	> 256	33.5
S, Susceptible rate (%); R, Resistant rate (%); I, intermediation rate (%); —, no criteria.																		

Levofloxacin had some antibacterial action to *Enterococcus faecalis*, and the resistance rate had declined slightly in the past decade (Table 9). The resistance rate of *Enterococcus faecium* kept in about 85%, and that of other fluoroquinolones, ampicillin and erythromycin were also above 85% (Table 10).

Table 9
Antimicrobial activity of antimicrobial agents against *Enterococcus faecalis* by year

Antibiotic	2009–2010(n = 111)			2011–2012(n = 276)			2013–2014(n = 311)			2015–2016(n = 332)			2017–2018(n = 342)		
	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R
Levofloxacin	128	62.2	36.9	64	69.2	29.0	64	65.3	31.2	64	66.6	31.6	64	73.1	25.7
Ciprofloxacin	NT	NT	NT	64	63.8	29.7	64	60.1	31.5	64	58.4	32.2	64	57.9	26.0
Moxifloxacin	32	63.1	32.4	16	72.5	26.1	16	70.1	28.9	16	69.0	29.5	16	74.9	24.0
Ampicillin	128	82.9	17.1	4	92.8	7.2	4	94.2	5.8	2	95.5	4.5	2	96.8	3.2
Erythromycin	> 256	4.5	77.5	> 256	2.5	76.4	> 256	10.3	72.0	> 256	7.2	67.5	> 256	5.6	67.3
HLGM	—	76.6	23.4	—	75.0	25.0	—	74.9	25.1	—	80.4	19.6	—	72.5	27.5
S, Susceptible rate (%); R, Resistant rate (%); HLGM, high concentration gentamicin; NT, not detected.															

Table 10
Antimicrobial activity of antimicrobial agents against *Enterococcus faecium* by year

Antibiotic	2009–2010(n = 193)			2011–2012(n = 299)			2013–2014(n = 343)			2015–2016(n = 348)			2017–2018(n = 357)		
	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R
Levofloxacin	128	14.5	84.5	128	11.0	86.0	128	9.9	86.6	128	10.6	85.1	128	10.6	84.3
Ciprofloxacin	NT	NT	NT	256	7.7	91.3	> 256	6.7	90.7	> 256	8.6	88.2	> 256	8.1	90.8
Moxifloxacin	32	14.0	83.4	32	8.4	87.0	32	7.9	88.9	64	10.6	86.2	64	9.2	88.2
Ampicillin	128	15.0	85.0	> 256	12.4	87.6	256	9.9	90.1	> 256	14.9	85.1	> 256	11.8	88.2
Erythromycin	> 256	3.6	88.6	> 256	1.7	91.3	> 256	3.8	87.8	> 256	3.4	83.9	> 256	4.8	84.3
HLGM	—	30.9	69.1	—	42.8	57.2	—	44.0	56.0	—	51.1	48.9	—	58.8	41.2
S, Susceptible rate (%); R, Resistant rate (%); HLGM, high concentration gentamicin; NT, not detected.															

Levofloxacin had a good antibacterial effect on *Streptococcus pneumoniae*, and the resistance rate was always lower than 3% (Table 11).

Table 11
Antimicrobial activity of antimicrobial agents against *Streptococcus pneumoniae* by year

Antibiotic	2009–2010(n = 352)			2011–2012(n = 369)			2013–2014(n = 340)			2015–2016(n = 290)			2017–2018(n = 300)		
	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R	MIC ₉₀	S	R
Levofloxacin	1	98.6	0.9	2	97.6	1.1	2	91.8	2.4	1	97.9	2.1	2	97.7	2.0
ciprofloxacin	NT	NT	NT	2	—	—	8	—	—	2	—	—	2	—	—
Moxifloxacin	0.25	99.1	0.9	0.5	98.1	1.9	0.5	98.2	1.2	0.25	97.9	1.4	0.25	98.3	1.3
Ceftriaxone	2	81.5	1.1	2	76.2	6.2	2	77.9	7.1	2	82.1	6.6	2	80.0	5.3
Cefepime	2	76.1	1.4	4	46.6	14.1	4	62.6	19.7	2	72.4	6.6	2	66.0	10.0
Imipenem	0.25	52.3	0.3	0.25	64.0	0.3	0.5	57.9	2.6	0.5	59.7	2.4	0.25	65.0	1.0
Meropenem	0.5	42.6	4.3	1	48.8	11.1	1	57.4	24.1	1	63.1	13.4	1	48.7	12.0
Azithromycin	> 256	1.7	97.4	> 256	0.3	98.1	> 256	2.1	97.4	> 256	2.8	96.2	> 256	1.7	97.0
S, Susceptible rate (%); R, Resistant rate (%); ceftriaxone and cefepime were determined by non meningitis standard (susceptibility: ≤1 mg / L; resistance: ≥4 mg / L); NT, not detected; —, no criteria.															

Discussion

The resistance rate of *E. coli* against fluoroquinolones has remained stable with occasionally decrease in the past decade, but it is still not less than 60%, which is at a high level. Due to the high proportion of ESBLs producing strains, the resistance rate of cephalosporins is also above 40%, and the resistance rates of ESBLs producing strains to fluoroquinolones and aminoglycosides were significantly higher than that of non ESBLs producing strains, and thus there was the high multiple drug resistance rate of clinical isolates of *E. coli* in China. There is no significant difference among different ages, wards and sample sources, which should be taken into account in the selection of treatment drugs. In recent years, the resistance rate of *Klebsiella pneumoniae* increased significantly because of the increase of carbapenem producing strains, which led to the increase of drug resistance rate of carbapenem and some β -lactam mixture. The resistance rate of fluoroquinolones also increased significantly in 2017–2018, which is positively related to the increase of carbapenem resistant bacteria, and its mechanism needs further study. The main reason for the decline of resistance rate of ESBLs producing strains in 2017–2018 and the significant increase of resistance rate of non ESBLs strains to fluoroquinolones, carbapenems and other drugs is that the carbapenem producing strains will be classified into non ESBLs strains because carbapenem conceals the detection of ESBLs phenotype. The impact of wrong classification was more and more significant with the increase of carbapenem producing strains. If these strains were classified as ESBLs producing bacteria, the resistance rates of ESBLs producing bacteria to levofloxacin and ciprofloxacin would be 66.9% and 80.6%, respectively. In addition, the resistance rate of *Klebsiella pneumoniae* isolated from ICU is significantly higher than that of non-ICU strains, which needs more attention.

The resistance rates of *Pseudomonas aeruginosa* against antibiotics were stable in recent ten years, while the resistance rates of *Acinetobacter baumannii* to all kinds of antibiotics tested increase to 65% – 75% from 2009 to 2014, which have been stable in high resistance level. *Acinetobacter baumannii* was the main bacteria causing nosocomial infection, and the resistance rates of strains isolated from ICU were higher, most of them were multi drug resistance or even pan drug resistance bacteria, so the combination of drugs should be considered in the treatment.

The decrease of resistance rate of *Staphylococcus aureus* to levofloxacin in recent ten years was related to the decrease of MRSA year by year. MRSA was a kind of multidrug-resistant bacteria, in addition to its resistance to β -lactams, its resistance to aminoglycosides, tetracyclines, macrolides and fluoroquinolones are significantly higher than MSSA. The levofloxacin resistance rate of *Staphylococcus aureus* strains isolated from sputum samples from ICU was higher. Levofloxacin has a good antibacterial activity against other Gram-positive bacteria such as *Streptococcus pneumoniae* and *Enterococcus faecalis*.

Since 2004, China began to strengthen the management of antibiotics. With the establishment of the National Antimicrobial clinical application and bacterial resistance monitoring network in 2005, a series of policies, regulations and technical specifications were issued to guide the rational application of antibiotics, which made the growth of bacterial resistance to some extent inhibited after 2007.

Conclusions

This ten-year retrospective analysis showed that levofloxacin basically maintained the original antibacterial activity to the bacteria in its antibacterial spectrum, and it still have a good antibacterial effect on the main pathogenic bacteria of community-acquired pneumonia, such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Klebsiella pneumoniae* and MSSA. The latest results were similar to other drug resistance monitoring reports in China [7] in the same period.

Abbreviations

MICs: antimicrobial minimum inhibitory concentrations; CLSI: Clinical and Laboratory Standards Institute; CAP: community acquired pneumonia; EUCAST: European Committee on Antimicrobial Susceptibility Testing; ESBLs: extended-spectrum β -lactamases; MRSA: Methicillin-resistant *Staphylococcus aureus*; MSSA: Methicillin-sensitive *Staphylococcus aureus*;

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LY conducted the design and was major contributor in writing the manuscript. GL collected the data and performed the statistical analysis. ZB and LY performed the anti-microbial susceptibility assay and data analysis. All authors read and approved the final manuscript.

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Figures

Figure 1

Cumulative percentage of levofloxacin against *E. coli* by year

Figure 2

Comparison of resistance rates of levofloxacin against *E. coli* from different category

Figure 3

Cumulative percentage of levofloxacin against *K. pneumoniae* by year

Figure 4

Comparison of resistance rates of levofloxacin against *K. pneumoniae* from different category

Figure 5

Cumulative percentage of levofloxacin against *A. baumannii* by year

Figure 6

Comparison of resistance rates of levofloxacin against *A. baumannii* from different category IMP-S: Imipenem susceptibility; IMP-NS: Imipenem non-susceptibility

Figure 7

Cumulative percentage of levofloxacin against *P. aeruginosa* by year

Figure 8

Comparison of resistance rates of levofloxacin against *P. aeruginosa* from different category IMP-S: Imipenem susceptibility; IMP-NS: Imipenem non-susceptibility

Figure 9

Cumulative percentage of levofloxacin against *S. aureus* by year

Figure 10

Comparison of resistance rates of levofloxacin against *S. aureus* from different category