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Fluoroquinolone antibiotics: in vitro antibacterial and time-kill bactericidal evaluation against etiology of bacteremia in human immunodeficiency virus (HIV)-infected patients

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Abstract

Background: Bacteremia constitutes a significant public health challenge and represents a vital cause of morbidity and mortality in HIV-infected patients, and fluoroquinolones are commonly prescribed antibiotics due to their range of activities and pharmacokinetic profiles. This study the evaluated antibacterial activities and time-kill kinetics of fluoroquinolone antibiotics: Ofloxacin (OFL), Ciprofloxacin (CIP) and Levofloxacin (LEV) against the etiology of bacteremia of genera *Staphylococcus, Streptococcus, Acinetobacter, Pseudomonas, Klebsiella, Haemophilus, Enterobacter*, and *Salmonella* using disc diffusion, micro-broth dilution and plate count techniques.

Results: The lowest mean growth inhibition zones (mm ± SD) of OFL, LEV, and CIP against the isolates were 10.5 ± 0.0, 10.1 ± 0.1 and 9.6 ± 0.3, respectively. The MIC values of OFL, LEV and CIP on isolates ranged from 6.25 to > 50 µg/mL, MBC ranged from 12.5 to > 50 µg/mL, while MBC/MIC ratios were ≤ 2. The time-kill assay revealed that logarithmic reductions in viable cell counts (Log_{10} CFU/mL) of bacteria exposed to OFL, LEV and CIP ranged from 0.17 to 2.14 for *P. aeruginosa*; 0.13 to 1.31 for *H. influenzae*; 0.04 to 2.23 for *Acinetobacter* spp; and 0.08 to 2.08 for *K. pneumoniae*. LEV and OFL (1 × MIC concentration) achieved bactericidal effects on *S. typhi* ST07 and *E. aerogenes* EA01 at 30 h post-inoculation, respectively, while ≥ 99.9% reduction in the number of viable *K. pneumoniae* cells exposed to CIP was achieved at 24 h post-inoculation.

Conclusion: The fluoroquinolones demonstrated higher inhibitory activities at higher concentrations against the etiology of bacteremia in HIV-infected patients, signifying a concentration-dependent inhibition of bacterial growth. The MIC-based time-kill curve analyses showed that LEV achieved 3 Log_{10}-fold reduction (≥ 99.9% reduction) in CFU/mL of most etiology of bacteremia faster compared with the other two fluoroquinolones.

Keywords: Fluoroquinolones, Serum bactericidal test, Time-kill kinetics, Bacteria, Cell viability

Background

The fluoroquinolones are a new class of broad-spectrum antimicrobial agents, synthetic fluorinated analogues of nalidixic acid with a 4-quinolone nucleus and a 1, 8-naphthyridone 3—carboxylic acid (Brar et al. 2020). The quinolone structure comprises a bicyclic system with

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a substituent at position N-1, a carboxyl group at position 3, a keto group at position 4, a fluorine atom at position 6 and a nitrogen heterocycle moiety at the C-7 position (Moshirfar et al. 2008). The fluoroquinolones offer first-rate activity against both aerobic Gram-negative bacteria (*Escherichia coli*, *Klebsiella* spp, *Pseudomonas aeruginosa*, and *Haemophilus influenzae*) and aerobic Gram-positive bacteria (*Nocardia* species, *Streptococcus pneumoniae, Enterococcus faecalis,* and *Staphylococcus aureus*) (Akinjogunla and Eghafona 2011; Akinjogunla et al. 2012). Similarly, some fluoroquinolones exhibit good activity against the most frequently isolated anaerobic bacteria such as *Peptostreptococcus, Fusobacterium*, *Bacteriodes* and *Prevotella* species (Goldstein et al. 2002; Snydman et al. 2002). The most extensively used fluoroquinolone antibiotic with potency against Gram-negative bacteria is Ciprofloxacin (Kocsis et al. 2016). Levofloxacin, a stereoisomer of ofloxacin, exerts a bactericidal effect against both Gram-negative and Gram-positive organisms (Kocsis et al. 2016).

The fluoroquinolones target bacterial DNA gyrase and topoisomerase IV enzymes that are essential for DNA replication and transcription (Akinjogunla and Eghafona 2011). DNA gyrase is a vital adenosine triphosphate (ATP)-hydrolyzing topoisomerase II enzyme that inhibits the detachment of gyrase from DNA and establishes negative super-helical twists in the bacterial DNA (Brar et al. 2020). Topoisomerase IV (Topo IV) is an A_2B_2 tetramer or a heterotetrameric structure consisting of two ParC subunits and two ParE subunits that are homologous to the two A subunits (gyrA) and two B subunits (gyrB) of DNA gyrase (Helgesen et al. 2021).

Fluoroquinolones are routinely used for the treatment of a variety of bacterial infections such as urinary tract infections and pyelonephritis, gastrointestinal and respiratory tract infections (Hooper 2000; Lode and Allewett 2002), skin and soft tissue infections (Martin and Zeigler 2004; Akinjogunla et al. 2012), cystic fibrosis (Akkerman-Nijland et al. 2021), prostatitis and osteomyelitis (Park et al. 2019), and uncomplicated sexually transmitted and bloodstream infections (Lo et al. 2017). Bacterial bloodstream infections constitute a significant public health challenge (Adeleye et al. 2010) and also cause a high morbidity and mortality rate in human immunodeficiency virus (HIV)—infected (Akinjogunla and Adegoke 2009; Ojo-Bola and Oluyege 2014). HIV-infected patients are prone to bloodstream infections due to altered B-cell function, defective cell-mediated immunity, and a dearth of neutrophils, leading to a rise in the susceptibility of patients to infections (Zurlo and Lane 1997).

Although reports on the susceptibility of bacterial isolates to fluoroquinolones have been documented, the studies on the time-kill bactericidal activities of fluoroquinolones against blood isolates from patients in our localities are not readily available. The objective of this study was to determine the in vitro antibacterial and time-kill bactericidal evaluation of Ofloxacin, Ciprofloxacin and Levofloxacin against the etiology of bacteremia in HIV-infected patients.

Methods

Materials used

Test tubes, test tube rack, conical flasks, sterile syringes, pipettes, Durham tubes, McCartney bottles, wire loops, Petri dishes, beaker, autoclave, incubator, oven, microscope slide, weighing balance, cotton wool, measuring cylinder, Bunsen burner, refrigerator and spectrophotometer were used.

Sterilization of materials

All glass wares used for this research were thoroughly washed with detergent and rinsed under clean running water. Thereafter, glass wares were sterilized in the hot air oven at 180 °C for an hour, and the wire loop was flamed to redness before and after use.

Collection and identification of etiology of bacteremia

The etiology of bacteremia in HIV-infected patients was identified by the Vitek 2 automated system (Biomerieux Inc., France) and as well by conventional biochemical tests. The results obtained were compared with databases for bacterial isolates in *Bergey's Manual of Systematic Bacteriology*, were used in this study (Holts et al. 1994). The isolates comprised of *Staphylococcus aureus* (n = 2), *Streptococcus pneumoniae* (n = 2), *Acinetobacter* spp (n = 1), *Salmonella typhi* (n = 2), *Klebsiella pneumoniae* (n = 2), *Enterobacter aerogenes* (n = 1), *Haemophilus influenzae* (n = 1), and *Pseudomonas aeruginosa* (n = 1). These isolates were obtained from the Microbiology Laboratory, University of Uyo, Akwa Ibom State.

Source of fluoroquinolone antibiotics

Ofloxacin (OFL 500 mg, Ronald Pharmaceuticals Pvt, Vadodara, India); Levofloxacin (LEV 500 mg, Zee Laboratory, India); and Ciprofloxacin (CIP 400 mg, Jiangsu Ruinian Pharmaceuticals Ltd, China) were purchased in tablet form from standard pharmacy stores in Uyo. Stock solutions (10 mg/mL) of OFL, LEV and CIP were prepared using sterile distilled water (dH_20) as the solvent and stored at 4 °C prior to each experiment.

Antibacterial activities of fluoroquinolones against etiology of bacteremia

The antibacterial activities of fluoroquinolone antibiotics: OFL, LEV and CIP against etiology of bacteremia in HIV-infected patients were determined by disc diffusion

method (CLSI 2018; Akinjogunla et al. 2021). The isolates used were *S. aureus* (SA08, SA21); *S. pneumoniae*, (SP02, SP10); *Acinetobacter* spp (AS01)*; S. typhi* (ST07, ST40); *K. pneumoniae* (KP26, KP32); *E. aerogenes* (EA01); *H. influenzae* (HI27) and *P. aeruginosa* (PA09). Mueller–Hinton agar (MHA) plates were aseptically prepared and 100 µL of each bacterial inoculum, prepared directly from an overnight nutrient agar plate and adjusted to 0.5 McFarland Turbidity Standard (corresponding to approximately 10^6 CFU/mL), was inoculated onto each MHA plate and thereafter evenly spread using a sterile spreader. Each test antibiotic (OFL, LEV and CIP) was dissolved in dH_20 to achieve graded concentrations of 2.5 and 5 mg/mL. Each sterile filter paper disc of 6 mm diameter was impregnated with 10 µL of 2.5 and 5 mg/mL test antibiotic. The impregnated discs were carefully placed on to MHA plates which had previously been inoculated with the isolates and were incubated at 37 °C for 24 h. A disc containing 10 µL of dH_20 that served as a solvent control was included in each plate. The same procedure described above was repeated for LEV and CIP. The experiments were performed in independent triplicates to validate the results, and the mean zones of inhibition diameter in millimeters were determined.

Evaluation of minimum inhibitory and minimum bacteriocidal concentrations of fluoroquinolones

The minimum inhibitory concentration (MIC) values of fluoroquinolone antibiotics: OFL, LEV and CIP against etiology of bacteremia in HIV-infected patients were determined using micro-broth dilution technique (CLSI 2018). Five hundred (500) mg of OFL, LEV and CIP were separately dissolved into 50 mL of dH_20 to give a concentration of 10 mg/mL. One milliliter (mL) of the stock solution (10 mg/mL of OFL, LEV and CIP) was serially diluted in sterile dH_20 by twofold dilution to achieve the range of test concentrations of 5—0.625 mg/mL for each antibiotic solution. To 100 µL of varying concentrations of OFL (0.625, 1.25, 2.5, 5 and 10 mg/mL) in test tubes was added nutrient broth (9.9 ml) to give the final concentrations of 6.25, 12.5, 25, 50 and 100 µg/mL for the MIC testing and a loopful of each prepared bacterial isolate was added. A tube comprising dH_20 with inoculum bacterial cells served as control. The same procedure described above was repeated for LEV and CIP. All the culture tubes were incubated at 37 °C for 24 h and thereafter the tubes were examined for microbial growth (turbidity measured using spectrophotometer). The MIC was taken as the lowest concentration of OFL, LEV and CIP that visibly inhibited the bacterial growth after 24 h of incubation.

For the minimum bacteriocidal concentration (MBC), the aliquot (1 mL) from each of MIC broth tubes without visible growth was inoculated onto each of the sterile nutrient agar plates using sterile pipette and streaked. The inoculated plates were inverted and incubated at 37 °C for 24 h. After incubation, the least concentration of the OFL that killed the bacterial isolate was observed and considered as the MBC value. The same procedure was repeated for LEV and CIP as described above.

Time-kill bactericidal evaluation of fluoroquinolones against etiology of bacteremia

The time-kill evaluation of fluoroquinolone antibiotics: OFL, LEV and CIP against etiology of bacteremia was carried out using macro broth dilution and pour plate techniques (CLSI 2018; Agbo et al. 2020). An overnight nutrient broth culture of each isolate, adjusted to 0.5 McFarland turbidity standard to obtain a starting inoculum between 10^5 and 10^6 CFU/mL (confirmed by quantitative plate counts), was used. The tubes containing the isolates were shaken at 150 rpm for 90 min at 37 °C to ensure that isolates were in their early exponential phase of growth. One (1) millilitre of each exponentially growing isolate was added to 9 ml of nutrient broth containing 1 mL of OFL (concentrations equal to MIC). Bacterial growth was quantified at time '0' h (immediately after addition of the OFL) and also at defined time intervals (6, 12, 18, 24 and 30 h) by aseptically taking 1 mL of aliquot, diluting serially (tenfold dilutions) in sterile dH_20 and plating out 1 mL of the final dilution onto a nutrient agar plate. All plates were incubated aerobically at 37 °C for 24 h and after incubation, the colonies on each plate were enumerated and viable cells were expressed as CFU/mL. The same procedure described above was repeated for LEV and CIP. A growth control comprising the inoculated broth medium without the antibiotics was set up, and 1 mL was plated on nutrient agar. The percent and log reductions of the bacterial cells exposed to OFL, LEV and CIP were calculated for each of the time intervals. The Log_{10} CFU/mL of survived bacterial cells against exposure time (hrs) were plotted on a semi-logarithm graph for each isolate to obtain time-kill curve. Activity of the antibiotics was considered bacteriocidal at the lowest concentration that reduced the initial inoculum by > 3 log_{10} CFU/mL (99.9%) and bacteriostatic at the lowest concentration that reduced the initial inoculum by < 3 log_{10} CFU/mL.

Reductions of the bacterial cells exposed to fluoroquinolone antibiotics

The percentage and logarithm reductions of the bacterial cells exposed to each antibiotic: OFL, LEV and CIP were, respectively, calculated as follows:

Table 1 Morphological and biochemical characteristics of etiology of bacteremia in HIV-infected patients

Gram reaction		COA	CAT	STA	VP	MR	NIT	IND	MOT	CIT	H_2S	OXI	OPS	FRU	XYL	RAF	MAN	MAL	GAL	LAC	GLU	SUC	Probable bacteria (codes)
Gram	Shape																						
+	Cocci	+	+	−	+	+	+	−	−	+	−	+	nd	−	−	−	+	+	+	+	+	+	*Staphylococcus aureus* SA08
+	Cocci	−	−	+	−	+	+	−	−	+	−	−	+	+	+	+	−	+	+	+	+	+	*Streptococcus pneumoniae* SP02
−	Rod	−	+	−	−	−	−	−	−	+	−	−	nd	−	+	+	−	+	+	−	+	−	*Acinetobacter* spp AS01
−	Rod	−	+	−	−	+	+	−	+	−	+	−	nd	+	+	−	+	+	+	+	+	−	*Salmonella typhi* ST07
−	Rod	−	+	−	+	−	+	−	−	+	−	−	nd	+	+	+	+	+	+	+	+	+	*Klebsiella pneumoniae* KP26
−	Rod	−	+	−	+	−	+	−	+	+	−	−	nd	+	+	+	+	+	+	+	+	+	*Enterobacter aerogenes* EA01
−	C-rod	−	+	−	−	−	+	+	−	+	−	+	nd	−	+	−	−	+	+	−	+	−	*Haemophilus influenzae* HI27
−	Rod	−	+	−	−	−	+	−	+	+	−	+	nd	−	−	−	+	−	−	−	−	−	*Pseudomonas aeruginosa* PA09
+	Cocci	+	+	−	+	+	+	−	−	+	−	+	nd	−	−	−	+	+	+	+	+	+	*Staphylococcus aureus* SA21
+	Cocci	−	−	+	−	+	+	−	−	+	−	−	+	+	+	+	−	+	+	+	+	+	*Streptococcus pneumoniae* SP10
−	Rod	−	+	−	−	+	+	−	+	−	+	−	nd	+	+	−	+	+	+	+	+	−	*Salmonella typhi* ST21
−	Rod	−	+	−	+	−	+	−	−	+	−	−	nd	+	+	+	+	+	+	+	+	+	*Klebsiella pneumoniae* KP32

COA Coagulase, *CAT* Catalase, *STA* Starch, *VP* Voges–Proskauer, *MR* Methyl red, *NIT* Nitrate, *IND* Indole, *MOT* Motility, *CIT* Citrate, H_2S Hydrogen sulphide, *OXI* Oxidase, *OPS* Optochin, *FRU* Fructose, *XYL* Xylose, *RAF* Raffinose, *MAN* Mannitol, *MAL* Maltose, *GAL* Galactose, *LAC* Lactose, *GLU* Glucose, *SUC* Sucrose, *nd* Not define

$$\text{Percentage (\%) reduction} = \left(\frac{\text{Initial counts} - \text{Counts at '}x\text{' interval}}{\text{Initial counts}}\right) \text{multiplied by 100}$$

$$\text{Logarithmic (Log) reduction} = \text{Log}_{10}(\text{Initial counts}) - \text{Log}_{10}(\text{Counts at '}x\text{' interval}).$$

Statistical analysis

All experiments were performed in triplicates, and statistical significance difference ($P < 0.05$) between the mean values was determined by Duncan multiple range test using Statistical Package for Social Sciences (SSPS version 22).

Results

Morphological and biochemical characteristics of etiology of bacteremia

The morphological and biochemical results of the bacterial isolates used for this study are.

presented in Table 1. The probable bacteria, re-identified by conventional biochemical tests and the Vitek 2 automated system, were *Staphylococcus aureus*, *Acinetobacter* spp., *Salmonella typhi*, *Streptococcus pneumoniae, Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Haemophilus influenzae,* and *Pseudomonas aeruginosa*.

Antibacterial activities of fluoroquinolone antibiotics against etiology of bacteremia

The LEV and CIP at a concentration of 5.0 mg/mL inhibited 100% of the tested isolates with the highest mean zone of growth inhibition of 19.3 ± 1.3 mm (Table 2). The results showed that OFL inhibited > 90% of these isolates (exception, *S. typhi* ST40) at a concentration of 5.0 mg/mL, while *Acinetobacter* spp. AS01; *S. pneumoniae* SP10 and *S. aureus* SA21 displayed resistance to growth inhibition of CIP at a concentration of 2.5 mg/mL. The lowest mean (mm ± SD) zone of inhibition obtained was 10.5 ± 0.0, 10.1 ± 0.1, and 9.6 ± 0.3 for 2.5 mg/mL of OFL, LEV and CIP, respectively. Additionally, 2.5 mg/mL of OFL had no antimicrobial activity against two tested isolates of *S. typhi* (ST07 and ST40) (Table 2).

Minimum inhibitory concentration and minimum bacteriocidal concentration of fluoroquinolones against etiology of bacteremia

The minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) values for OFL, LEV, and CIP against the etiology of bacteremia in HIV-infected patients are shown in Table 3. The MIC values of OFL ranged from the lowest (12.5 µg/mL) for *K. pneumoniae* KP32 and KP26; *E. aerogenes* EA01 and *S. pneumoniae* SP02 to the highest (> 50 µg/mL) for *S. typhi* ST40. Six isolates had a Levofloxacin MIC value of 12.5 µg/mL, and 50.0% of the isolates had a Levofloxacin MIC value of 25 µg/mL. The MIC values of CIP for the isolates ranged from 12.5 to 50 µg/mL, while *P. aeruginosa* PA09 showed a Ciprofloxacin MIC value of 6.25 µg/mL. The MBC values of LEV and CIP for the 12 isolates: *S. aureus* (n = 2), *S. pneumoniae* (n = 2), *Acinetobacter* spp. (n = 1), *S. typhi* (n = 2), *K. pneumoniae* (n = 2), *E. aerogenes* (n = 1), *H. influenzae* (n = 1), and *P. aeruginosa* (n = 1) ranged from 12.5 to 50 µg/mL, while MBC values of OFL for the isolates were between the ranges of 12.5 to > 50 µg/mL. The MBC/MIC ratios of OFL, LEV and CIP on the isolates ranged between 1 and 2.

Time-kill bactericidal evaluation of fluoroquinolones against etiology of bacteremia

The percentage and logarithmic reductions of viable bacterial cells (Log_{10} CFU/ml) exposed to OFL, LEV, and CIP at 6 h intervals after incubation are presented in Table 4. Bacteriocidal activity of OFL, LEV, and CIP was deemed to be present if there was a ≥ 99.9% reduction in survival from the original inoculum. The results indicated that OFL, LEV, and CIP exhibited a reduction in the viable cell counts of the test bacteria after 30 h of interaction at the 1 × MIC concentrations. The percent and log reduction in viable cell counts of *P. aeruginosa* PA09 exposed to OFL ranged from 54.8 to ≥ 99.9% and 0.35 to 2.14 Log_{10} CFU/ml after 30 h of interaction, respectively. The time-kill kinetics curves of OFL against *P. aeruginosa* PA09 and *H. influenzae* HI27 are shown in Fig. 1. The lowest log reduction in viable cell counts of *H. influenzae* HI27, *Acinetobacter* spp. AS01 and *K. pneumoniae* KP32 exposed to OFL was 0.3, 0.04 and 0.08 Log_{10} CFU/ml, respectively. The percent reduction in viable cell counts of *S. typhi* ST07 and *E. aerogenes* EA01 exposed to OFL ranged from 16.0 to 96.8% and 23.1 to ≥ 99.9% after 30 h of interaction, respectively. The ranges of log reduction in viable cell counts of *S. aureus* SA21 and *S. pneumoniae* SP02 exposed to OFL for 30 h were 0.1 to 1.28 Log_{10} CFU/ml and 0.19 to 1.26 Log_{10} CFU/ml, respectively. Figure 1 also depicts the time-kill kinetics curve of.

OFL against *S. aureus* SA21 and *S. pneumoniae* SP02.

The log reduction in viable cell counts of *P. aeruginosa* PA09, *H. influenzae* HI27, *Acinetobacter* spp. AS01 and *K. pneumoniae* KP32 exposed to LEV for 30 h ranged from 0.17 to 1.58; 0.23 to 2.0; 0.08 to 1.35 and 0.67 to 2.04 Log_{10} CFU/mL, respectively (Table 4). The time-kill kinetics curve of LEV (1 × MIC) and control against *S. typhi* ST07,

Table 2 Antibacterial activities of fluoroquinolone antibiotics against etiology of bacteremia in HIV-infected patients

Bacterial isolates	Isolates code	Zone of inhibition (mm ± SD)						
		Ofloxacin		Levofloxacin		Ciprofloxacin		10%
		2.5 mg/mL	5.0 mg/mL	2.5 mg/mL	5.0 mg/mL	2.5 mg/mL	5.0 mg/mL	DMSO
P. aeruginosa	PA09	15.1 ± 0.1^a	17.5 ± 0.5^a	13.5 ± 0.1^a	16.4 ± 1.0^a	15.7 ± 0.2^a	18.0 ± 1.0^a	NZ
H. influenzae	HI27	11.8 ± 0.2^a	14.3 ± 0.2^b	14.0 ± 0.0^a	16.5 ± 0.0^a	13.3 ± 0.2^a	15.7 ± 0.1^a	NZ
Acinetobacter spp	AS01	NZ	12.5 ± 0.1	11.2 ± 0.3^a	13.8 ± 0.2^a	NZ	11.2 ± 0.0	NZ
K. pneumoniae	KP32	12.8 ± 0.1^a	16.1 ± 0.2^b	12.1 ± 0.1^a	15.7 ± 0.5^b	11.0 ± 0.0^a	14.4 ± 0.2^b	NZ
K. pneumoniae	KP26	12.3 ± 0.2^a	16.8 ± 0.5^b	14.9 ± 0.2^a	19.3 ± 1.3^b	14.2 ± 0.2^a	19.0 ± 1.5^b	NZ
S. typhi	ST07	NZ	9.3 ± 0.1	10.3 ± 0.1^a	13.2 ± 0.1^a	NZ	11.5 ± 0.1	NZ
S. typhi	ST40	NZ	NZ	9.5 ± 0.0^a	11.2 ± 0.2^a	NZ	10.0 ± 0.0	NZ
E. aerogenes	EA01	14.6 ± 0.4^a	18.0 ± 1.0^b	14.7 ± 0.5^a	17.5 ± 1.0^b	12.0 ± 0.0^a	16.4 ± 0.3^b	NZ
S. aureus	SA08	10.5 ± 0.0^a	15.5 ± 0.5^b	12.4 ± 0.2^a	16.0 ± 0.5^b	12.9 ± 0.1^a	15.7 ± 0.2^a	NZ
S. aureus	SA21	NZ	12.0 ± 0.0	10.1 ± 0.1^a	14.4 ± 0.1^b	NZ	12.0 ± 0.0	NZ
S. pneumoniae	SP02	11.1 ± 0.1^a	16.9 ± 0.1^b	9.6 ± 0.3^a	14.6 ± 0.1^b	13.6 ± 0.4^a	17.2 ± 0.2^b	NZ
S. pneumoniae	SP10	NZ	13.3 ± 0.2	11.5 ± 0.2^a	16.5 ± 0.5^a	13.9 ± 1.1^a	19.0 ± 1.0^b	NZ

Each value represents the mean of three replicates and standard deviation. Mean within the column followed by the different superscript letters are significant as determined by Duncan's multiple range test ($P < 0.05$)

mm Means, *SD* Standard deviation, *NZ* No inhibitory zone, *DMSO* Dimethyl sulphoxide

Table 3 Minimum inhibitory and minimum bacteriocidal concentrations of fluoroquinolone antibiotics against etiology of bacteremia in HIV-infected patients

Bacterial isolates	Isolate codes	MIC (µg/mL)			MBC (µg/mL)			MBC/MIC ratios		
		OFL	LEV	CIP	OFL	LEV	CIP	OFL	LEV	CIP
P. aeruginosa	PA09	6.25	12.5	6.25	12.5	25	12.5	2	2	2
H. influenzae	HI27	25	12.5	12.5	50	12.5	25	2	1	2
Acinetobacter spp	AS01	50	25	50	50	50	50	1	2	1
K. pneumoniae	KP32	12.5	12.5	25	25	25	50	2	2	2
K. pneumoniae	KP26	12.5	12.5	12.5	25	12.5	12.5	2	1	1
S. typhi	ST07	50	25	50	50	25	50	1	1	1
S. typhi	ST40	> 50	25	50	> 50	50	50	1	2	1
E. aerogenes	EA01	12.5	12.5	12.5	12.5	12.5	25	1	1	2
S. aureus	SA08	25	12.5	12.5	25	25	25	1	2	2
S. aureus	SA21	50	25	50	50	50	50	1	2	1
S. pneumoniae	SP02	12.5	25	12.5	25	50	25	2	2	2
S. pneumoniae	SP10	50	25	12.5	50	50	25	1	2	2

MIC Minimum inhibitory concentration, *MBC* minimum bacteriocidal concentration, *OFL* Ofloxacin, *LEV* Levofloxacin, *CIP* Ciprofloxacin

E. aerogenes EA01, *S. aureus* SA21 and *S. pneumoniae* SP02 are shown in Fig. 1. At a 1 × MIC concentration, LEV achieved bactericidal effects on *S. typhi* ST07 and *S. pneumoniae* SP02 at 30 h post-inoculation, while ≥ 99.9% reduction in survival from the original inoculum was achieved for *S. aureus* SA21 at 24 h post-inoculation (Table 4). The percentage and logarithm reductions of viable bacterial cells (Log_{10} CFU/mL) exposed to CIP at 6 h intervals after incubation are presented in Table 4. The CIP had bactericidal effects on *P. aeruginosa* PA09, *H. influenzae* HI27 and *Acinetobacter* spp. AS01 at 30 h post-inoculation, while ≥ 99.9% reduction in survival from the original inoculum was achieved for *K. pneumoniae* KP32 at 24 h post-inoculation. Also, CIP was not bactericidal against *S. typhi* ST07, *E. aerogenes* EA01, *S. aureus* SA21 and *S. pneumoniae* SP02 at 1.0 times the MIC. The time-kill kinetics curve of CIP (1 × MIC) and control against bacterial isolates is shown in Fig. 1.

Table 4 Percentage and logarithmic reductions in bacterial cells exposed to fluoroquinolone antibiotics

Isolate codes	Exposed time (h)	OFL			LEV			CIP		
		PC (CFU/ml)	Log_{10} CFU/ml	%/Log reduction	PC (CFU/ml)	Log_{10} (CFU/ml)	%/Log reduction	PC (CFU/ml)	Log_{10} CFU/ml	%/Log reduction
PA09	0	4.6×10^5	5.66	NA/NA	4.0×10^5	5.60	NA/NA	4.1×10^5	5.61	NA/NA
	6	5.0×10^4	4.70	89.1/0.97	4.9×10^4	4.69	87.8/0.91	5.0×10^4	4.70	87.8/0.91
	12	2.5×10^3	3.40	95.0/1.3	1.3×10^3	3.11	97.3/1.58	3.2×10^3	3.51	93.6/1.39
	18	3.1×10^2	2.49	87.6/0.9	2.9×10^2	2.46	77.7/0.65	3.0×10^2	2.48	90.6/1.03
	24	1.4×10^2	2.15	54.8/0.35	1.5×10^2	2.18	48.3/0.29	1.2×10^2	2.08	60.0/0.4
	30	NG	0.0	$\geq$ 99.9/2.14	1.0×10^2	2.0	33.3/0.17	NG	0.0	$\geq$ 99.9/2.08
	0	5.1×10^5	5.71	NA/NA	4.5×10^5	5.65	NA/NA	4.0×10^5	5.60	NA/NA
	6	2.6×10^5	5.41	49.0/0.3	5.3×10^4	4.72	88.2/0.93	5.6×10^4	4.75	86/0.85
HI27	12	6.0×10^4	4.78	76.9/0.63	3.1×10^4	4.49	41.5/0.23	4.1×10^3	3.61	92.7/1.14
	18	4.3×10^3	3.63	92.8/1.15	2.0×10^3	3.30	93.5/1.19	2.9×10^2	2.46	92.9/1.15
	24	2.1×10^2	2.32	95.1/1.31	1.0×10^2	2.0	95.0/1.3	1.0×10^2	2.0	65.5/0.46
	30	1.0×10^2	2.0	52.4/0.32	NG	0.0	$\geq$ 99.9/2.0	NG	0.0	$\geq$ 99.9/2.0
AS01	0	3.9×10^5	5.59	NA/NA	3.6×10^5	5.56	NA/NA	3.0×10^5	5.48	NA/NA
	6	2.8×10^4	4.45	92.8/1.14	2.0×10^4	4.30	94.4/1.26	4.1×10^4	4.61	86.3/0.87
	12	1.6×10^3	3.20	94.3/1.25	3.4×10^3	3.53	83.0/0.77	2.6×10^3	3.41	93.7/1.2
	18	1.1×10^2	2.04	93.1/1.16	1.5×10^2	2.18	95.6/1.35	2.0×10^2	2.30	92.3/1.11
	24	1.0×10^2	2.0	90.9/0.04	1.2×10^2	2.08	20/0.1	1.7×10^2	2.23	15/0.07
	30	NG	0.0	$\geq$ 99.9/2.0	1.0×10^2	2.0	16.7/0.08	NG	0.0	$\geq$ 99.9/2.23
KP32	0	6.9×10^5	5.84	NA/NA	5.4×10^5	5.73	NA/NA	5.4×10^5	5.73	NA/NA
	6	1.1×10^5	5.04	84.1/1.26	6.0×10^4	4.78	88.9/0.95	4.8×10^4	4.68	91.1/1.05
	12	1.0×10^4	4.0	90.9/0.77	3.5×10^3	3.54	94.2/1.24	2.5×10^3	3.40	94.8/1.28
	18	3.6×10^3	3.56	64.0/1.35	5.1×10^2	2.71	85.4/0.83	1.2×10^2	2.08	95.2/1.32
	24	1.7×10^2	2.23	95.3/0.1	1.1×10^2	2.04	78.4/0.67	NG	0.0	$\geq$ 99.9/2.08
	30	1.0×10^2	2.0	41.2/0.08	NG	0.0	$\geq$ 99.9/2.04	NG	0.0	$\geq$ 99.9/0.0
ST07	0	3.3×10^5	5.52	NA/NA	3.7×10^5	5.57	NA/NA	3.1×10^5	5.49	NA/NA
	6	4.7×10^4	4.67	85.8/0.85	2.9×10^4	4.46	92.2/1.11	4.2×10^4	4.62	86.5/0.87
	12	1.5×10^3	3.18	96.8/1.49	2.5×10^3	3.40	91.4/1.06	1.5×10^3	3.18	96.4/1.44
	18	2.5×10^2	2.40	83.3/0.78	1.9×10^2	2.28	92.4/1.12	2.9×10^2	2.46	80.7/0.72
	24	2.1×10^2	2.32	16.0/0.08	1.0×10^2	2.0	47.4/0.28	2.4×10^2	2.38	17.2/0.08
	30	1.0×10^2	2.0	52.4/0.32	NG	0.0	$\geq$ 99.9/2.0	1.5×10^2	2.18	37.5/0.2

Table 4 (continued)

Isolate codes	Exposed time (h)	OFL			LEV			CIP		
		PC (CFU/ml)	Log_{10} CFU/ml	%/Log reduction	PC (CFU/ml)	Log_{10} (CFU/ml)	%/Log reduction	PC (CFU/ml)	Log_{10} CFU/ml	%/Log reduction
EA01	0	4.5×10^5	5.65	NA/NA	5.7×10^5	5.76	NA/NA	4.0×10^5	5.60	NA/NA
	6	3.4×10^4	4.53	92.4/1.13	2.6×10^4	4.41	95.4/1.35	2.9×10^5	5.46	27.5/0.14
	12	2.6×10^3	3.41	92.1/1.12	3.6×10^3	3.56	86.2/0.85	4.5×10^4	4.65	84.5/0.81
	18	2.0×10^3	3.30	23.1/0.11	2.4×10^2	2.38	93.3/1.18	3.1×10^3	3.49	93.1/1.16
	24	1.7×10^2	2.23	91.5/1.07	2.0×10^2	2.30	16.7/0.08	2.0×10^2	2.30	93.5/1.19
	30	NG	0.0	$\geq$ 99.9/2.23	1.1×10^2	2.04	45.0/0.26	1.4×10^2	2.15	30.0/0.15
SA21	0	3.7×10^5	5.57	NA/NA	5.0×10^5	5.70	NA/NA	4.6×10^5	5.66	NA/NA
	6	4.0×10^4	4.60	89.2/0.97	3.9×10^4	4.59	92.2/1.11	4.0×10^4	4.60	91.3/1.06
	12	2.1×10^3	3.32	94.8/1.28	2.0×10^3	3.30	94.9/1.29	2.5×10^4	4.40	37.5/0.20
	18	2.5×10^2	2.40	88.1/0.92	1.7×10^2	2.23	91.5/1.07	3.9×10^3	3.59	84.4/0.81
	24	2.0×10^2	2.30	20.0/0.1	NG	0.0	$\geq$ 99.9/2.23	2.6×10^2	2.41	93.3/1.18
	30	1.6×10^2	2.20	20.0/0.1	NG	0.0	$\geq$ 99.9/0.0	1.5×10^2	2.18	42.3/0.23
SP02	0	4.1×10^5	5.61	NA/NA	3.8×10^5	5.58	NA/NA	4.0×10^5	5.60	NA/NA
	6	1.0×10^5	5.00	75.6/0.61	6.5×10^4	4.81	82.9/0.77	1.0×10^5	5.00	75.0/0.6
	12	6.3×10^4	4.80	37.0/0.20	4.1×10^3	3.61	93.7/1.20	5.5×10^4	4.74	45.0/0.26
	18	3.5×10^3	3.54	94.4/1.26	2.7×10^2	2.43	93.4/1.18	3.4×10^3	3.53	93.8/1.21
	24	2.0×10^2	2.30	94.3/1.24	1.5×10^2	2.18	44.4/0.25	2.5×10^2	2.40	92.6/1.13
	30	1.3×10^2	2.11	35.0/0.19	NG	0.0	$\geq$ 99.9/2.18	1.0×10^2	2.00	60.0/0.40

PA09, *P. aeruginosa*; HI27, *H. influenzae*; AS01, *Acinetobacter* spp; KP32, *K. pneumonia*; PC, Plate counts; CFU, Colony forming units; ml, Millilitre; NG, No growth; NA, Not available; OFL, Ofloxacin; LEV, Levofloxacin; CIP, Ciprofloxacin; ST07, *S. typhi*; EA01, *E. aerogenes*; SA21, *S. aureus*; SP02, *S. pneumoniae*; PC, Plate Counts; CFU, Colony forming units; ml, Millilitre; NG, No growth; NA, Not available; OFL, Ofloxacin; LEV, Levofloxacin; CIP, Ciprofloxacin

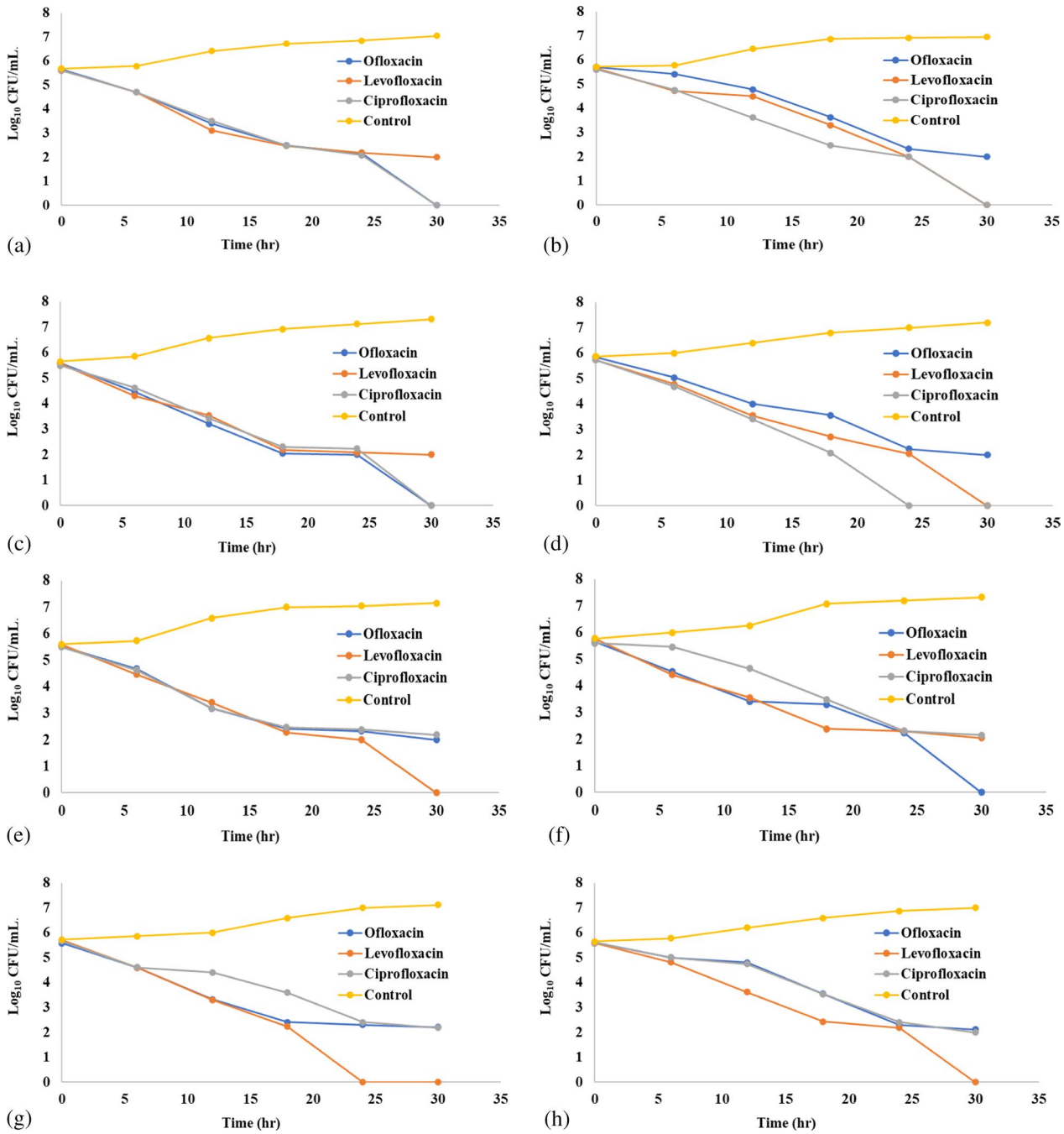

Fig. 1 Time-kill kinetics curve of Ofloxacin, Levofloxacin, Ciprofloxacin (1 × MIC) and Control against **a** *P. aeruginosa* PA09, **b** *H. influenzae* HI27, **c** *Acinetobacter* spp AS01, **d** *K. pneumoniae* KP32, **e** *S. typhi* ST07 **f** *E. aerogenes* EA01 **g** *S. aureus* SA21 **h** *S. pneumoniae* SP02

Increases in viable cell counts of bacteria not exposed to OFL, LEV, and CIP within the 30 h of incubation period were observed and are presented in Table 5. The increase in viable cell counts of *P. aeruginosa* PA09, *H. influenzae* HI27, *Acinetobacter* spp. AS01 and *K. pneumoniae* KP32 ranged from 5.67 to 7.04, 5.72 to 6.95, 5.65 to 7.30, and 5.87 to 7.20 (Log_{10} CFU/mL), respectively. Similarly, an increase in viable cell counts from 5.60 to 7.15 Log_{10} CFU/mL was observed for *S. typhi* ST07; 5.77 to 7.32 Log_{10} CFU/mL for *E. aerogenes* EA01; 5.72 to 7.11 Log_{10} CFU/mL for *S. aureus* SA21 and 5.65 to 7 Log_{10} CFU/mL for *S. pneumoniae* SP02.

Table 5 Growth of bacterial cells unexposed to fluoroquinolone antibiotics

Bacterial isolates	Codes	Time interval (h)	Plate counts (CFU/ml)	Log $_{10}$ (CFU/ml)
P. aeruginosa	PA09	0	4.8×10^5	5.68
		6	6.2×10^5	5.79
		12	2.6×10^6	6.41
		18	5.3×10^6	6.72
		24	7.0×10^6	6.84
		30	1.1×10^7	7.04
H. influenzae	HI27	0	5.3×10^5	5.72
		6	5.9×10^5	5.77
		12	2.9×10^6	6.46
		18	7.4×10^6	6.87
		24	8.3×10^6	6.92
		30	9.0×10^6	6.95
Acinetobacter spp	AS01	0	4.5×10^5	5.65
		6	7.0×10^5	5.85
		12	3.6×10^6	6.56
		18	8.1×10^6	6.91
		24	1.3×10^7	7.11
		30	2.0×10^7	7.30
K. pneumoniae	KP32	0	7.4×10^5	5.87
		6	1.0×10^6	6.00
		12	2.5×10^6	6.40
		18	6.2×10^6	6.79
		24	1.0×10^7	7.00
		30	1.6×10^7	7.20
S. typhi	ST07	0	4.0×10^5	5.6
		6	5.4×10^5	5.73
		12	3.8×10^6	6.58
		18	1.0×10^7	7.00
		24	1.1×10^7	7.04
		30	1.4×10^7	7.15
E. aerogenes	EA01	0	5.9×10^5	5.77
		6	1.0×10^6	6.0
		12	1.8×10^6	6.26
		18	1.2×10^7	7.08
		24	1.6×10^7	7.20
		30	2.1×10^7	7.32
S. aureus	SA21	0	5.2×10^5	5.72
		6	7.2×10^5	5.86
		12	1.0×10^6	6.00
		18	3.9×10^6	6.59
		24	1.0×10^7	7.00
		30	1.3×10^7	7.11
S. pneumoniae	SP02	0	4.5×10^5	5.65
		6	6.0×10^5	5.78
		12	1.6×10^6	6.20
		18	3.8×10^6	6.58
		24	7.4×10^6	6.87
		30	1.0×10^7	7.00

Discussion

Bacterial bloodstream infections have constituted a significant public health challenge and have represented a vital cause of morbidity and mortality in HIV-infected patients (Adeyemi et al. 2010). Fluoroquinolones are constantly prescribed antibiotics owing to their range of activities and pharmacokinetic profiles (Grillon et al. 2020). The present study provides fundamental information on the in vitro antibacterial activities and time-kill bactericidal evaluation of three fluoroquinolone antibiotics: CIP, OFL and LEV against *S. aureus*, *S. pneumoniae*, *Acinetobacter* spp, *S. typhi*, *K. pneumoniae, E. aerogenes, H. influenzae* and *P. aeruginosa* from blood samples of HIV-infected patients. In vitro antibacterial activities of fluoroquinolone antibiotics against *H. influenzae, S. pneumoniae* and *S. typhi* in our study are consistent with the reports of Mascellino et al. (1998) and Akinjogunla and Eghafona (2011) on activities of fluoroquinolones on clinical bacterial isolates. Comparably, activity of CIP against Gram-negative bacterial isolates corresponds to the findings of Kumar et al. (2002) that CIP exhibited antibacterial activities against *P. aeruginosa, Salmonella* spp. and *K. pneumoniae*. The fluoroquinolone antibiotics used in this study demonstrated higher inhibitory activities at 5 mg mL^{-1} concentration against bacterial isolates than at 2.5 $mgmL^{-1}$ concentration, signifying a concentration-dependent inhibition of bacterial growth. Relatedly, several reports have shown that fluoroquinolone antibiotics are concentration-dependent inhibition medications (Wrights et al. 2000; Pham et al. 2019).

In our study, OFL at a concentration of 5.0 $mgmL^{-1}$ had no inhibitory effect on the growth of *S. typhi* and this confirms the previous findings of Aliyu et al. (2021)

on time-kill analysis of OFL against *S. typhi.* The weakened activity of OFL against *Salmonella* spp, indicating an acquired gene for Ofloxacin resistance, has been reported (Kariuki et al. 2015). Generally, OFL is administered either orally or intravenously for effective treatment of a wide range of infections, and its primary mechanism of action is to inhibit bacterial DNA gyrase (Todd and Faulds 1991). *S. pneumoniae* and *H. influenzae* displayed sensitivity to LEV, even at a low concentration of 2.5 mg mL^{-1}. This is in line with the report by Zhang et al. (2019) on the high susceptibility of group B streptococci to LEV. Additionally, the sensitivity of *H. influenzae* and *S. pneumoniae* to LEV corresponds to the previous report by Akinjogunla and Eghafona (2011) on the susceptibility of *S. pneumoniae* and *H. influenzae* to LEV. However, this is contrary to Bastida et al. (2003) who reported a high rate of LEV resistant *H. influenzae.* Levofloxacin has been reported to be effective against *H. influenzae* (Anderson and Perry 2008). Levofloxacin promotes the breakage of DNA strands by inhibiting DNA gyrase in susceptible organisms which causes inhibition of the relaxation of supercoiled DNA (Podder and Sadiq 2021).

The MIC values for fluoroquinolones against 12 isolates from HIV-infected patients ranged from 6.25 to > 50 µg/mL. Levofloxacin and Ciprofloxacin MIC values for *P. aeruginosa* PA09 were 12.5 and 6.25 µg/mL respectively, indicating that Levofloxacin had higher MIC values than CIP for *P. aeruginosa*. This agrees with MacGowan et al. (1999) that LEV had higher MIC values than CIP and was less bactericidal at equivalent concentrations against *P. aeruginosa*. Relatedly, Ciprofloxacin MIC values for *S. pneumoniae* SP02 and SP10 were lower than those of Levofloxacin MIC values. These findings agree with Ramakrishnan et al. (2010) who obtained Ciprofloxacin MIC values lower than that of LEV in their studies, and this also confirms a high degree of activity of CIP against *S. pneumoniae*. We obtained MBC/MIC ratios of 1:1 and 1:2. Noviello et al. (2002) also reported MBC/MIC ratio in the range 1:1 and 1:2 in their study on comparative in vitro bacteriostatic and bactericidal activity of LEV and CIP. The bactericidal activities of fluoroquinolones against bacterial isolates from HIV-infected patients were determined using a time-kill kinetics assay. Bacteriocidal activity of fluoroquinolones was deemed to be present if there was a 3 Log_{10}-fold reduction in CFU/mL of surviving bacteria or a ≥ 99.9% reduction in survival from the original inoculum. Our study showed that fluoroquinolone antibiotics exhibited ≥ 99.9% reductions in some viable cell counts of the test bacteria between 24 and 30 h of interaction at (1 × MIC) concentrations. We also observed that LEV and CIP displayed a 3 Log_{10}-fold reduction in CFU/mL of *K. pneumoniae.* This is contrary to Grillon et al. (2020) who in their time-kill studies reported an absence of bactericidal activity of LEV and CIP against *K. pneumoniae*. Ciprofloxacin had bactericidal effects on *P. aeruginosa* PA09 at 30 h post-inoculation, and this agrees with Segatore et al. (2020) who reported the bactericidal activity of CIP on different phenotypes of *P. aeruginosa.* A marked reduction in the viable cell counts of *H. influenzae* HI27, *S. pneumoniae* SP02 and *S. typhi* ST07 exposed to LEV at (1 × MIC) concentrations was observed, but ≥ 99.9% reduction was obtained at 30 h post-inoculation.

The result is slightly dissimilar to the findings of Kitzis et al. (1999) who obtained a ≥ 99.9% reduction in *H. influenzae* at 18 h of exposure to LEV. The percent reduction in viable cell counts of *E. aerogenes* EA01 and *Acinetobacter* spp AS01 exposed to OFL ranged from 23.1 to ≥ 99.9% and 90.9 to ≥ 99.9% after 30 h of interaction, respectively. Our results on the time-kill kinetics of OFL against *Acinetobacter* spp AS01 are comparable with the previous findings of Sato et al. (1996) who reported a high bactericidal action of OFL and the related new quinolone agents against *Acinetobacter* spp. and other clinical bacterial isolates.

Conclusions

The CIP, OFL and LEV demonstrated higher inhibitory activities at higher concentrations.

against etiology of bacteremia in HIV-Infected patients, signifying a concentration-dependent inhibition of bacterial growth. In terms of MIC and MBC values, CIP was the most active drug against *S. pneumoniae* and LEV against *S. typhi, S. aureus* and *Acinetobacter* spp. The MIC-based time-kill curve analyses showed that LEV achieved a 3 Log_{10}-fold reduction (≥ 99.9% reduction) in CFU/mL of most bacteria tested quicker compared with the other two fluoroquinolones. Consequent upon these findings, in vivo antibacterial studies of OFL, LEV, and CIP on different experimental animals with bacterial bloodstream infections are required.

Abbreviations

OFL: Ofloxacin; LEV: Levofloxacin; CIP: Ciprofloxacin; MHA: Mueller–Hinton agar; MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; PC: Plate count.

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Author contributions

This work was carried out in collaboration between all the authors. OJA and ATO were involved in experimental design. UFU and IE performed the statistical analysis. OJA, UEU and ATO drafted the manuscript and managed literature

searches. MFA, JE and EKA proofread the draft manuscript and made major revisions. All authors have read and approved the final manuscript.

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Competing interests
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