



Dichotomous selection of high-level oxacillin resistance in by fluoroquinolones

Axel Dalhoff, Sabine Schubert

► To cite this version:

Axel Dalhoff, Sabine Schubert. Dichotomous selection of high-level oxacillin resistance in by fluoroquinolones. International Journal of Antimicrobial Agents, 2010, 36 (3), pp.216. 10.1016/j.ijantimicag.2010.04.014 . hal-00608993

HAL Id: hal-00608993

<https://hal.science/hal-00608993>

Submitted on 17 Jul 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Dichotomous selection of high-level oxacillin resistance in *Staphylococcus aureus* by fluoroquinolones

Authors: Axel Dalhoff, Sabine Schubert

PII: S0924-8579(10)00233-5
DOI: doi:10.1016/j.ijantimicag.2010.04.014
Reference: ANTAGE 3331



To appear in: *International Journal of Antimicrobial Agents*

Received date: 13-8-2009
Revised date: 29-4-2010
Accepted date: 30-4-2010

Please cite this article as: Dalhoff A, Schubert S, Dichotomous selection of high-level oxacillin resistance in *Staphylococcus aureus* by fluoroquinolones, *International Journal of Antimicrobial Agents* (2008), doi:10.1016/j.ijantimicag.2010.04.014

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Dichotomous selection of high-level oxacillin resistance in *Staphylococcus aureus* by fluoroquinolones

Axel Dalhoff *, Sabine Schubert

*University Hospital Schleswig-Holstein, Campus Kiel, Institute for Infection
Medicine, Brunswiker Str. 4, 24105 Kiel, Germany*

ARTICLE INFO

Article history:

Received 13 August 2009

Accepted 30 April 2010

Keywords:

Staphylococcus aureus

Fluoroquinolones

High-level oxacillin resistance

Dichotomous selection

* Corresponding author. Tel.: +49 202 265 5236; fax: +49 202 265 5297.

E-mail address: adalhoff@t-online.de (A. Dalhoff).

ABSTRACT

The objective of this study was to determine whether exposure of *Staphylococcus aureus* to early (ciprofloxacin or levofloxacin) and a recent fluoroquinolone (moxifloxacin) has differential potential as a mutator or selector for meticillin resistance. The potential of fluoroquinolones to act as mutators or selectors was studied in 24 strains each of healthcare-associated meticillin-susceptible *S. aureus* (MSSA) and meticillin-resistant *S. aureus* (MRSA) as well as 6 strains of community-acquired MRSA. Mutator or selector potential was studied first by exposing isolates to 0.5× the fluoroquinolone minimal inhibitory concentration (MIC) and screening for either single-step fluoroquinolone resistance or high-level oxacillin resistance; second, by exposing the heteroresistant MRSA P8 parent strain as well as fluoroquinolone-resistant subpopulations derived from strain P8 to constant fluoroquinolone concentrations ranging from 0.015 mg/L to 128 mg/L; and third, by exposing the heteroresistant MRSA population of strain P8 to fluctuating concentrations of ciprofloxacin, levofloxacin and moxifloxacin simulating oral doses of 500 mg twice a day, 500 mg once daily (qd) and 400 mg qd, respectively, compared with amoxicillin/clavulanic acid 500 mg three times a day. Total viable counts and subpopulations resistant to 2×, 4× and 8× the fluoroquinolone MICs and to 32, 64 and 128 mg/L oxacillin [high-level oxacillin (hl-OXA)-resistant] were quantitated. None of the fluoroquinolones acted as a mutator; ciprofloxacin and levofloxacin selected for hl-OXA resistance, whereas moxifloxacin selected towards hl-OXA resistance by one order of magnitude less frequently. The P8 parent and fluoroquinolone-resistant subpopulations were eliminated by ciprofloxacin or levofloxacin

concentrations >10-fold higher than the MICs, whereas moxifloxacin eliminated all subpopulations by concentrations 2–3-fold the MIC. Finally, exposure of P8 to fluctuating amoxicillin/clavulanic acid, ciprofloxacin and levofloxacin concentrations, respectively, caused a rapid selection of fluoroquinolone and hl-OXA resistance. Moxifloxacin reduced total viable counts rapidly, thus preventing the emergence of resistant subpopulations. In conclusion, fluoroquinolones do not act as mutators towards hl-OXA resistance. However, ciprofloxacin and levofloxacin are potent selectors of hl-OXA resistance, whereas moxifloxacin is a poor selector. In contrast to ciprofloxacin and levofloxacin, moxifloxacin exerts a high bactericidal activity against staphylococci, thus minimising the probability for selection of resistance. Thus, fluoroquinolones exert a dichotomous MRSA-selective potential in heteroresistant MRSA.

1. Introduction

Meticillin resistance leading to treatment failure was detected 1 year after its launch [1,2]. Shortly after the identification of meticillin resistance, it became apparent that 'resistant clones' were not true mutants but represented a minority population of a genetically uniform culture [3]. It is characteristic for most meticillin-resistant *Staphylococcus aureus* (MRSA) that expression of meticillin resistance is heterogeneous; most cells express a low level of resistance, from which a small subpopulation of highly resistant subclones segregate at a frequency of 10^{-7} to 10^{-3} [4]. Therefore, phenotypic expression of meticillin resistance is highly variable and each strain has a characteristic profile of meticillin resistance heterogeneity [5]. The mechanism of meticillin resistance is due to the acquisition of an altered penicillin-binding protein 2A (PBP2A) [1,6] encoded by the *mecA* gene, which is located on the so-called staphylococcal cassette chromosome *mec* (SCC*mec*) also containing additional genes for antibiotic resistance elements encoding for aminoglycoside, tetracycline and macrolide–lincosamide–streptogramin B (MLS_B) resistance, insertion sequences as well as genes of uncertain function [4,7]. Therefore, these drug classes can act as co-selectors for MRSA.

A steady rise in the proportion of *S. aureus* resistant to meticillin occurred in the 1960–1970s, but inexplicably the numbers fell between the latter half of the 1970s and the early 1980s. The reasons for this decline are not clear. On the one hand there was a continuous and moderately heavy use of antibiotics such as isoxazolyl penicillins (flucloxacillin, nafcillin, dicloxacillin, etc.), which have been implicated as a selective pressure for MRSA. On the other hand,

aminoglycosides to which many MRSA were susceptible at that time were increasingly prescribed. In the 1980s, a rise in gentamicin-resistant MRSA was reported. Not only do aminoglycosides act as co-selectors for MRSA [8–12] but use of cephalosporins, penicillins, carbapenems, macrolides, vancomycin, metronidazole and fluoroquinolones has been important in selecting for MRSA [13–16]. Although a causal relationship between antimicrobial drug use and MRSA colonisation and/or infection has been demonstrated [17–19], the evidence is not conclusive.

In any consideration of the selective effect of antibiotic regimens, not only the resistance mechanisms of each antibiotic/organism combination should be considered. It is also of clinical relevance if any agent of a given drug class may exert the same or differential selective pressure. Furthermore, the problem of co-selection of strains has to be considered, as some of the *SCCmec* islands contain genes encoding for aminoglycoside, tetracycline and *MLS_B* resistance [7]. In addition, selective pressures in the hospital environment are very complex as different intensities of care are used for different patients [14–16] and different policies for hospital cleaning and hygienic measures are applied [20,21].

A further complication is the recent recognition that some community-acquired (CA)-MRSA may originate in hospitals and that novel strains of MRSA can evolve by horizontal gene transfer of the *mecA* gene and then be subjected to selective pressure.

Although antibacterials in general select for MRSA, fluoroquinolones in particular are considered to act as mutators or selectors for meticillin resistance in *S. aureus* [22,23]. Therefore, three questions to be addressed are: is fluoroquinolone use per se linked to the emergence of MRSA? Do fluoroquinolones act as selectors of or mutators for meticillin resistance in *S. aureus*? And if so, may different fluoroquinolones be characterised by a differential selective potential?

2. Materials and methods

2.1. Strains, media and agents studied

Twenty-four strains each of healthcare-associated (HA) meticillin-susceptible *S. aureus* (MSSA) and MRSA as well as six CA-MRSA were studied. All strains were shown to be negative or positive for *mecA* by hybridisation. All strains had recently been isolated and characterised at the University Hospitals in Muenster (Germany) and Zürich (Switzerland). Fluoroquinolone-resistant MRSA were generated in the laboratory as described previously [24]. Briefly, six HA-MRSA were grown overnight in brain–heart infusion (BHI) broth (Oxoid, Wesel, Germany) containing doubling dilutions of either ciprofloxacin, levofloxacin or moxifloxacin. From the culture containing the highest drug concentration permitting visible bacterial growth [i.e. 0.5× the minimal inhibitory concentration (MIC)], an aliquot was transferred as a 1:20 dilution to inoculate a second set of serial fluoroquinolone dilutions. Following overnight incubation, the dilution procedure was repeated again until the initial fluoroquinolone MIC increased eight-fold. MICs were determined by the agar

dilution method according to Clinical and Laboratory Standards Institute guidelines [25]. BHI agar was inoculated with 1×10^5 colony-forming units (CFU)/mL and was incubated for 20 h at 36 ± 1 °C in ambient air.

Ciprofloxacin and moxifloxacin were provided by Bayer Healthcare (Leverkusen, Germany). Levofloxacin (Sanofi-aventis Deutschland GmbH, Frankfurt, Germany) and amoxicillin/clavulanic acid (fixed 4:1 combination; GlaxoSmithKline GmbH & Co. KG, Munich, Germany) were obtained from commercially available sources. All agents were of defined activity.

2.2. Spontaneous emergence of meticillin or fluoroquinolone resistance

Single-step fluoroquinolone resistance testing was performed as outlined by Dalhoff et al. [24]. To characterise the spontaneous emergence of fluoroquinolone resistance, each of the 24 MSSA and MRSA strains (inoculum 1×10^9 – 10^{10} CFU/mL) were spread over BHI agar plates incorporating the study drugs (ciprofloxacin, moxifloxacin or levofloxacin) at $4 \times$ MIC of the individual test organisms. Following overnight incubation at 36 ± 1 °C, the frequency of spontaneous resistance was determined by colony counting.

Emergence of high-level oxacillin (hl-OXA) resistance in the 24 strains each of MSSA and MRSA was tested in the presence of $0.5 \times$ MIC of the three fluoroquinolones as well as amoxicillin (positive control) and saline (negative control) by incorporating 128 mg/L oxacillin into agar containing 2% NaCl. The

resultant oxacillin-resistant subpopulations can be defined as *hl*-MRSA populations because of the high oxacillin concentration used for selection of MRSA.

2.3. Population analysis profiling

One heteroresistant MRSA strain (*S. aureus* P8) was used for these experiments. From this strain, fluoroquinolone-resistant subpopulations resistant to 2 \times , 4 \times and 8 \times the MIC of either ciprofloxacin (initial MIC = 0.25 mg/L), levofloxacin (initial MIC = 0.25 mg/L) or moxifloxacin (initial MIC = 0.125 mg/L) were elicited by incubating ca. 10 log₁₀ CFU/mL in tubes containing rising concentrations of either fluoroquinolone. From the culture containing the highest drug concentration permitting visible growth following an incubation period of 48 h, an aliquot was transferred as a 1:20 dilution to inoculate a second set of serial fluoroquinolone dilutions. After 48 h incubation, the dilution procedure was repeated again until subpopulations were elicited that were resistant to the multiples of the initial individual fluoroquinolone MICs.

The parent strain and the three either ciprofloxacin-, levofloxacin- or moxifloxacin-resistant subpopulations were grown to ca. 9 log₁₀ CFU/mL in BHI broth for 20 h at 37 °C. The final inoculum of ca. 11 log₁₀ CFU/mL was obtained by centrifugation and re-suspension of the test strain in fresh medium. Aliquots were then plated onto BHI agar plates containing a fluoroquinolone concentration range (one fluoroquinolone concentration per plate) of 0.015, 0.025, 0.03, 0.05, 0.06, 0.075, 0.10, 0.12, 0.15, 0.20, 0.24,

0.30, 0.40, 0.48, 0.60, 0.80, 0.96, 1.2, 1.6, 2.4, 3.2, 3.8, 4.8, 6.4, 7.7, 9.6, 12.8, 15.5, 19.2, 25.6, 31.0, 38.4, 51.0, 62.0, 76.8, 102.4 and 128 mg/L.

Plates were incubated in ambient air at 36 ± 1 °C for 20 h. Thus, MRSA strain P8 was exposed to 37 fluoroquinolone concentrations. Total viable counts were quantitated on drug-free agar plates as well as on plates containing 2% NaCl and 128 mg/L oxacillin. Plates were controlled after 24 h, but colonies were counted after an incubation period of 48 h at 37 °C (as colonies were not clearly visible after 24 h). Population analysis profiling was used to determine whether the three different fluoroquinolones studied exhibited a differential bactericidal activity against three different fluoroquinolone-resistant subpopulations of a heteroresistant MRSA indicator strain.

2.4. In vitro pharmacokinetic simulation model

One heteroresistant MRSA strain (P8) was used for these experiments. A slightly modified one-compartment model according to and illustrated by Grasso et al. [26] was used. Briefly, this model consists of a central compartment (calibrated flasks only were used for all experiments) that is placed on heated magnetic stirrer. The antibacterial is pumped via a programmable pump into the central compartment until the maximum serum concentration (C_{\max}) to be simulated is reached at T_{\max} . Thereafter, drug-free medium is pumped by a second programmable pump into the central compartment and is continuously eliminated in parallel to mimic elimination half-life ($t_{1/2}$) values. Control growth in the absence of antibiotics is studied in the same model under identical conditions. To avoid wash-out of bacteria from the central compartment when simulating fluctuating concentration

profiles of agents with short half-lives (e.g. amoxicillin $t_{1/2}$ = 3.5 h; ciprofloxacin $t_{1/2}$ = 5.0 h), bacteria were entrapped in dialysis tubing (neoLab, Heidelberg, Germany) fixed within the central compartment. Antibacterials selectively diffuse into the tubing whereas bacteria do not penetrate into the surrounding medium. Bacteria were not washed-out from the central compartment provided the simulated $t_{1/2}$ values exceeded 8 h (e.g. levofloxacin and moxifloxacin) [27].

Bacteria were grown in BHI broth and were incubated in ambient air at 37 °C. Viable counts were determined at 0, 0.5, 1, 2, 3, 5, 7, 12, 24, 25, 26, 27, 29, 36, 48, 49, 50, 51, 53, 55, 60, 72, 73, 74, 75, 77, 79, 84 and 96 h. The impact of fluoroquinolone or amoxicillin/clavulanic acid carry-over on viable counts was minimised first by diluting the samples and second by plating the aliquots on cation-enriched agar or *Bacillus cereus* β -lactamase-containing agar, thus inactivating the agents. At the point of quantification of viable counts, antibiotic concentrations were measured by a conventional cup-plate agar diffusion test with *Bacillus subtilis* spore suspension as the indicator organism. Drug concentrations were monitored in the absence and presence of bacteria. Actual drug concentrations achieved in this model were on average within 4.2% of the desired profile, which is in agreement with previous studies [28]. Interday and intraday reproducibility ranged from 92.4% to 98.5% as qualified calibrated flasks were used.

The following pharmacokinetic (PK) parameters calculated on the basis of total drug concentrations were used as a basis for the PK simulations: for

amoxicillin 500 mg three times a day, $C_{\max} = 11.0$ mg/L, $T_{\max} = 2.0$ h, $t_{1/2} = 3.5$ h, area under the concentration–time curve (AUC) = 55.4 mg h/L and protein binding (PB) = 20% [29,30]; for ciprofloxacin 500 mg twice a day, $C_{\max} = 2.5$ mg/L, $T_{\max} = 1.5$ h, $t_{1/2} = 5$ h, AUC = 22.3 mg h/L and PB = 30% [31]; for levofloxacin 500 mg once daily (qd), $C_{\max} = 6.6$ mg/L, $T_{\max} = 1.3$ h, $t_{1/2} = 8.0$ h, AUC = 57.3 mg h/L and PB = 30% [31]; and for moxifloxacin 400 mg qd, $C_{\max} = 3.3$ mg/L, $T_{\max} = 1.5$ h, $t_{1/2} = 13$ h, AUC = 30 mg h/L and PB = 40% [31,32]. Calculated free serum concentrations (amoxicillin $C_{\max} = 9.5$ mg/L and AUC = 44.4 mg h/L; ciprofloxacin $C_{\max} = 2.0$ mg/L and AUC = 16.1 mg h/L; levofloxacin $C_{\max} = 4.8$ mg/L and AUC = 40.5 mg h/L; and moxifloxacin $C_{\max} = 2.3$ and AUC = 19.8 mg h/L) simulated in the in vitro pharmacodynamic model are illustrated in Fig. 1.

Viable counts were quantitated by spreading the samples onto agar plates containing *B. cereus* β -lactamase (Merck KGaA, Darmstadt, Germany) or 5 mM calcium to inactivate amoxicillin or the fluoroquinolones. In parallel, samples were plated first onto agar plates containing 2 \times , 4 \times and 8 \times the MIC of the fluoroquinolone studied; and second, samples were plated onto agar plates containing 2% NaCl + 32, 64 and 128 mg/L oxacillin and incubated at 35 °C. Thus, the emergence of fluoroquinolone-resistant subpopulations as well as the emergence of MRSA heteroresistance is addressed in parallel in these experiments.

3. Results

MRSA strains in the strict sense (*mecA*-positive but fluoroquinolone susceptible) are as susceptible to the three fluoroquinolones studied as MSSA strains (Table 1). Furthermore, the spontaneous mutation frequencies towards fluoroquinolone resistance were almost the same amongst the CA-MRSA, HA-MRSA and MSSA strains studied as well as the three laboratory-generated fluoroquinolone-resistant MRSA strains and the fluoroquinolone-resistant strain P8 (Table 1). Strains with 4× the initial moxifloxacin MIC could be elicited in <50% of the isolates studied, whereas ciprofloxacin or levofloxacin resistance emerged in all isolates studied (Table 1).

High-level meticillin resistance emerged amongst the strains studied at a low frequency. Pre-existing highly meticillin-resistant subclones emerged spontaneously in the HA-MRSA but not the CA-MRSA controls at a frequency of $1-5 \times 10^{-7}$ and were selected by amoxicillin/clavulanic acid at a frequency of $1-2 \times 10^{-2}$ to 2.4×10^{-4} .

Ciprofloxacin and levofloxacin selected for high-level meticillin resistance by one to two orders of magnitude and moxifloxacin by three to four orders of magnitude less frequently than amoxicillin/clavulanic acid (Table 1).

Selection of highly meticillin-resistant subclones by fluoroquinolones has been studied by population analysis profiling. The indicator MRSA strain P8 and its laboratory-generated fluoroquinolone-resistant mutants were exposed to a broad range of fluoroquinolone concentrations and total viable counts were

quantitated as well as the frequency with which highly meticillin-resistant subclones (128 mg/L oxacillin) emerged upon exposure to any of the fluoroquinolone concentrations (Table 2). For the sake of clarity, emergence of highly meticillin-resistant subclones upon exposure to 1, 5, 10, 50 and 100 mg/L of either ciprofloxacin, levofloxacin or moxifloxacin only are summarised in Table 2.

Exposure of the indicator strain *S. aureus* P8 to a broad range of constant ciprofloxacin and levofloxacin concentrations resulted in a moderately concentration-dependent reduction of the initial inoculum. The inoculum of the parent population of *S. aureus* P8 was eradicated by 12.8 mg/L ciprofloxacin; the inoculum of the subpopulations resistant to 2× the ciprofloxacin MIC was eradicated by 38.4 mg/L ciprofloxacin; whereas the inoculated viable counts of the subpopulations being resistant to 4× and 8× the MIC were reduced by ca. 9.2 and 6.0 log CFU/mL by 128 mg/L ciprofloxacin.

Likewise, the inoculum of the parent population of *S. aureus* P8 was eradicated by 3.8 mg/L levofloxacin; the inocula of the subpopulations resistant to 2× and 4× the levofloxacin MICs were eradicated by 12.8 mg/L and 31.0 mg/L levofloxacin, respectively; whereas the inoculated viable counts of the subpopulation resistant to 8× the MIC was reduced by ca. 10.4 log CFU/mL by 128 mg/L levofloxacin. In contrast, moxifloxacin eradicated the inocula of the parent strain as well as the three moxifloxacin-resistant subpopulations at moxifloxacin concentrations of 0.30, 0.60, 1.2 and 2.4 mg/L, respectively.

The frequencies with which highly meticillin-resistant subclones emerged decreased in parallel with the reduction of total viable counts by increasing fluoroquinolone concentrations (Table 2). Ciprofloxacin selected more highly meticillin-resistant subclones than levofloxacin; moxifloxacin exerted no selective potential.

The MRSA selective potential of fluoroquinolones was analysed in an in vitro PK simulation model. Again, the MRSA heteroresistant strain P8 served as the indicator strain. Fluctuating amoxicillin/clavulanic acid concentrations, serving as a control, selected rapidly for resistance (Fig. 2). Ciprofloxacin rapidly selected both the ciprofloxacin-resistant (within 24 h) as well as the three oxacillin-resistant subpopulations. CFUs of the three pre-existing hi-MRSA subpopulations (32, 64 and 128 mg/L oxacillin) remained constant for ca. 36 h. Thereafter, a continuous increase in CFUs of the oxacillin-resistant subpopulations was noted (Fig. 3). Levofloxacin was also a rapid selector of fluoroquinolone resistance. The three oxacillin-resistant subpopulations were selected from 48 h onwards and less rapidly than by ciprofloxacin (Fig. 4). Moxifloxacin, however, eliminated all the hi-MRSA subpopulations of *S. aureus* P8, i.e. the fluoroquinolone-resistant as well as the heteroresistant MRSA subpopulations, within 36 h, so that the in vitro PK simulation model was sterilised (Fig. 5).

4. Discussion

Data generated in this study demonstrate that fluoroquinolones do not act as mutators towards *hl*-OXA resistance. However, ciprofloxacin and levofloxacin are potent selectors, whereas moxifloxacin is a poor selector. Thus, fluoroquinolones exert a dichotomous MRSA-selective potential in heteroresistant MRSA. This finding is in good agreement with data generated by Venezia et al. [33]. Fluoroquinolone-susceptible heteroresistant MRSA were exposed to constant subinhibitory concentrations of ciprofloxacin, levofloxacin, gatifloxacin and moxifloxacin. All four fluoroquinolones selected for *hl*-OXA resistance; however, the proportion of *hl*-OXA-resistant MRSA was one to three orders of magnitude greater following ciprofloxacin exposure than upon exposure to the two 8-methoxy fluoroquinolones [33]. Furthermore, the MICs of the strains studied in this series of experiments, the mutational frequencies and the bactericidal activities are in good agreement with previously published data [34–38].

The low MRSA-selective potential of moxifloxacin in contrast to the high selective potential of ciprofloxacin and levofloxacin is likely due to the high and pronounced bactericidal activity of moxifloxacin against MSSA and MRSA, whereas ciprofloxacin and levofloxacin reduce viable counts of *S. aureus* much more slowly as described in this study and as published previously [17,38–40].

Clinical use of early quinolones like levofloxacin as opposed to the use of recent quinolones like moxifloxacin in the treatment of Gram-positive

infections in indications such as community-acquired respiratory tract infections or skin and skin-structure infections may trigger the selection of quinolone-resistant MRSA (see above and [15,33,41]). Furthermore, MRSA tended to develop fluoroquinolone resistance more frequently than MSSA (Table 1), which is in agreement with previous data [33]. This phenomenon may be due to the fact that on the chromosomal map of the *S. aureus* genome the *mecA* gene is located between protein A and DNA gyrase genes. Therefore, mutations in the gyrase may have an effect on the expression of *mecA* in heteroresistant MRSA strains [33] and some cell wall-associated proteins such as protein A and fibronectin-binding proteins [42,43].

In conclusion, the experimental data of this study and the clinical findings [15,33,41] discussed above support the notion that quinolone resistance in staphylococci is not a class effect. The propensities for resistance development in vitro as well as in the clinical setting are dissociated, with ciprofloxacin and levofloxacin being better selectors of meticillin resistance and better drivers of quinolone resistance than the newer fluoroquinolones that have enhanced anti-Gram-positive activity. Thus, the increasing use of generic fluoroquinolones or inappropriately dosed levofloxacin in the treatment of community-acquired respiratory tract infections purely for economic reasons may jeopardise the beneficial clinical efficacy of the modern fluoroquinolones. Prescription guidelines should also consider the dissociated propensities for resistance development.

Funding

None.

Competing interests

None declared.

Ethical approval

Not required.

Accepted Manuscript

References

- [1] Jevons MP. Celbenin-resistant staphylococci. *Br Med J* 1961;1:124–5.
- [2] Knox R. Celbenin-resistant staphylococci. *Br Med J* 1961;1:126.
- [3] Lacey RW. The mechanism of methicillin resistance. *J Antimicrob Chemother* 1986;18:435–9.
- [4] Berger-Bächi B, Rohrer S. Factors influencing methicillin resistance in staphylococci. *Arch Microbiol* 2002;178:165–71.
- [5] Tomasz A, Nachmann S, Leaf H. Stable classes of phenotypic expression in methicillin-resistant clinical isolates of staphylococci. *Antimicrob Agents Chemother* 1991;35:124–9.
- [6] Utsui Y, Yokota T. Role of an altered penicillin-binding protein in methicillin- and cephem-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1985;28:397–403.
- [7] Deresinski S. Methicillin-resistant *Staphylococcus aureus*: an evolutionary, epidemiologic, and therapeutic odyssey. *Clin Infect Dis* 2005;40:562–73.
- [8] Johnson AP, Pearson A, Duckworth G. Surveillance and epidemiology of MRSA bacteraemia in the UK. *J Antimicrob Chemother* 2005;56:455–62.
- [9] Marples RR, Reith S. Methicillin-resistant *Staphylococcus aureus* in England and Wales. *Commun Dis Rep CDR Rev* 1992;2:R25–9.
- [10] Shanson DC. Antibiotic-resistant *Staphylococcus aureus*. *J Hosp Infect* 1981;2:11–36.
- [11] Hawkey PM. The growing burden of antimicrobial resistance. *J Antimicrob Chemother* 2008;62(Suppl 1):i1–9.

- [12] Reynolds LA, Tansey EM, editors. *Superbugs and superdrugs: a history of MRSA*, Vol. 32. London, UK: Wellcome Trust Centre for the History of Medicine at UCL; 2008.
- [13] Johnson AP, Aucken HM, Cavendish S, Ganner M, Wale MC, Warner M, et al. Dominance of EMRSA-15 and -16 among MRSA causing nosocomial bacteraemia in the UK: analysis of isolates from the European Antimicrobial Resistance Surveillance System (EARSS). *J Antimicrob Chemother* 2001;48:143–4.
- [14] Dziekan G, Hahn A, Thüne K, Schwarzer G, Schäfer K, Daschner FD, et al. Methicillin-resistant *Staphylococcus aureus* in a teaching hospital: investigation of nosocomial transmission using a matched case–control study. *J Hosp Infect* 2000;46:263–70.
- [15] Weber SG, Gold HS, Hooper DC, Karchmer AW, Carmeli Y. Fluoroquinolones and the risk for methicillin-resistant *Staphylococcus aureus* in hospitalised patients. *Emerg Infect Dis* 2003;9:1415–22.
- [16] Graffunder EM, Venezia RA. Risk factors associated with nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) infection including previous use of antimicrobials. *J Antimicrob Chemother* 2002;49:999–1005.
- [17] Salgado CD, Farr BM, Calfee DP. Community-acquired methicillin-resistant *Staphylococcus aureus*: a meta-analysis of prevalence and risk factors. *Clin Infect Dis* 2003;36:131–9.
- [18] Harbarth S, Frankhäuser C, Schrenzel J. Universal screening for methicillin-resistant *Staphylococcus aureus* at hospital admission and nosocomial infection in surgical patients. *JAMA* 2008;299:1149–57.

- [19] Tacconelli E, DeAngelis G, Cataldo MA, Pozzi E, Cauda R. Does antibiotic exposure increase the risk of methicillin-resistant *Staphylococcus aureus* (MRSA) isolation? A systematic review and meta-analysis. J Antimicrob Chemother 2008;61:26–38.
- [20] Dancer SJ. Importance of the environment in methicillin-resistant *Staphylococcus aureus* acquisition: the case for hospital cleaning. Lancet Infect Dis 2008;8:101–13.
- [21] Dancer SJ. The role of environmental cleaning in the control of hospital-acquired infection. J Hosp Infect 2009;73:378–85.
- [22] Crowcroft NS. Methicillin-resistant *Staphylococcus aureus* and antimicrobial use in Belgian hospitals. Infect Control Hosp Epidemiol 1999;20:31–6.
- [23] MRSA-Bedrohung durch Antibiotikatherapie? NLGA aktuell 2004;3:1–2.
- [24] Dalhoff A, Schubert S, Ullmann U. Effect of pH on the in vitro activity of and propensity for emergence of resistance to fluoroquinolones, macrolides, and a ketolide. Infection 2005;33(Suppl 2):36–43.
- [25] Clinical and Laboratory Standards Institute. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard*. 7th ed. Document M7-A7. Wayne, PA: CLSI; 2006.
- [26] Grasso S, Meinardi G, deCarneri I, Tamassia V. New in vitro model to study the effect of antibiotic concentration and rate of elimination on antibacterial activity. Antimicrob Agents Chemother 1978;13:570–6.
- [27] Dalhoff A. Pharmacodynamics of fluoroquinolones. J Antimicrob Chemother 1999;43(Suppl B):51–9.

- [28] Schubert S, Dalhoff A, Stass H, Ullmann U. Pharmacodynamics of moxifloxacin and levofloxacin simulating human serum and lung concentrations. *Infection* 2005;33(Suppl 2):15–21.
- [29] Dalhoff A, Koeppe P, von Kobyletzki D. Studies on the pharmacokinetics of amoxicillin after intravenous, intramuscular and oral administration [in German]. *Arzneimittelforschung* 1981;31:1148–57.
- [30] Todd PA, Benfield P. Amoxicillin/clavulanic acid. An update of its antibacterial activity, pharmacokinetic properties and therapeutic use. *Drugs* 1990;39:264–307.
- [31] Dudley MN. Pharmacokinetics of fluoroquinolones. In: Hooper DC, Rubinstein E, editors. *Quinolone antimicrobial agents*. 3rd ed. Washington, DC: ASM Press; 2003. p. 115–32.
- [32] Stass H, Kubitz D. Pharmacokinetics and elimination of moxifloxacin after oral and intravenous administration in man. *J Antimicrob Chemother* 1999;43(Suppl B):83–90.
- [33] Venezia RA, Domaracki BE, Evans AM, Preston KE, Graffunder EM. Selection of high-level oxacillin resistance in heteroresistant *Staphylococcus aureus* by fluoroquinolones exposure. *J Antimicrob Chemother* 2001;48:375–81.
- [34] Dalhoff A, Schmitz FJ. In vitro antibacterial activity and pharmacodynamics of new quinolones. *Eur J Clin Microbiol Infect Dis* 2003;22:203–21.
- [35] Thauvin-Eliopoulos C, Eliopoulos GM. Activity in vitro of the quinolones. In: Hooper DC, Rubinstein E, editors. *Quinolone antimicrobial agents*. 3rd ed. Washington, DC: ASM Press; 2003. p. 91–111.

- [36] Schmitz FJ, Higgins PG, Mayer S, Fluit AC, Dalhoff A. Activity of quinolones against Gram-positive cocci: mechanisms of drug action and bacterial resistance. *Eur J Clin Microbiol Infect Dis* 2002;21:647–59.
- [37] Goldstein EJC, Citron DM, Warren YA, Tyrrell KL, Rybak MJ. Virulence characteristics of community-associated *Staphylococcus aureus* and in vitro activities of moxifloxacin alone and in combination against community-associated and healthcare-associated methicillin-resistant and -susceptible *S. aureus*. *J Med Microbiol* 2008;57:452–56.
- [38] Dalhoff A, Petersen U, Endermann R. In vitro activity of BAY 12-8039, a new 8-methoxyquinolone. *Chemotherapy* 1996;42:410–25.
- [39] Schaper KJ, Schubert S, Dalhoff A. Kinetics and quantification of antibacterial effects of β -lactams, macrolides and quinolones against Gram-positive and Gram-negative RTI pathogens. *Infection* 2005;33(Suppl 2):3–14.
- [40] Kayser FH, Santanam P, Huf E. Comparative activity of moxifloxacin and other quinolones against staphylococci [in German]. *Chemother J* 2000;9:114–9.
- [41] Graffunder E, Venezia RA. Risk factors associated with nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) infection including previous use of antimicrobials. *J Antimicrob Chemother* 2002;49:999–1005.
- [42] Bisognano C, Vaudaux PE, Lew DP, Ng EYW, Hooper DC. Increased expression of fibronectin-binding proteins by fluoroquinolone-resistant *Staphylococcus aureus* exposed to subinhibitory levels of ciprofloxacin. *Antimicrob Agents Chemother* 1997;41:906–13.

- [43] Bisognano C, Vaudaux P, Rohner P, Lew DP, Hooper DC. Inhibition of fibronectin-binding proteins and increased adhesion of quinolone-resistant *Staphylococcus aureus* by subinhibitory levels of ciprofloxacin. *Antimicrob Agents Chemother* 2000;44:1428–37.

Accepted Manuscript

Fig. 1. Measured drug concentrations representing the free fraction following oral administration of amoxicillin 500 mg three times a day (●), ciprofloxacin 500 mg twice a day (◆), levofloxacin 500 mg once daily (▲) and moxifloxacin 400 mg once daily (□)

Fig. 2. Effect of fluctuating amoxicillin/clavulanic acid (AMC) concentrations simulating oral doses of 500 mg three times a day against the heteroresistant methicillin-resistant *Staphylococcus aureus* (MRSA) strain P8. ◆, total viable counts; ■, AMC-resistant subpopulations with 2× MIC; ▲, AMC-resistant subpopulations with 4× MIC; and ●, 8× MIC. Viable counts of drug-free controls (not shown) increased to $2-3 \times 10^8$ CFU/mL, 10^9 CFU/mL and 5×10^9 CFU/mL at $t = 6$ h, 12 h and >24 h. MIC, minimal inhibitory concentration; CFU, colony-forming units.

Fig. 3. Effect of fluctuating ciprofloxacin concentrations simulating oral doses of 500 mg twice a day against the heteroresistant methicillin-resistant *Staphylococcus aureus* (MRSA) strain P8. ■, total viable counts; solid lines, fluoroquinolone-resistant subpopulations with 2× MIC (◆), 4× MIC (●) and 8× MIC (*) elevated ciprofloxacin MICs; and dotted lines, high-level oxacillin-resistant MRSA (hl-MRSA) subpopulations with oxacillin (OXA) MICs of 32 mg/L (▲), 64 mg/L (×) and 128 mg/L (+). Viable counts of drug-free controls (not shown) increased to $2-3 \times 10^8$ colony-forming units (CFU)/mL, 10^9 CFU/mL and 5×10^9 CFU/mL at $t = 6$ h, 12 h and >24 h. MIC, minimal inhibitory concentration; CFU, colony-forming units.

Fig. 4. Effect of fluctuating levofloxacin concentrations simulating oral doses of 500 mg once daily against the heteroresistant meticillin-resistant *Staphylococcus aureus* (MRSA) strain P8. Symbols as in Fig. 3. MIC, minimal inhibitory concentration; CFU, colony-forming units.

Fig. 5. Effect of fluctuating moxifloxacin concentrations simulating oral doses of 400 mg once daily against the heteroresistant meticillin-resistant *Staphylococcus aureus* (MRSA) strain P8. Symbols as in Fig. 3. MIC, minimal inhibitory concentration; CFU, colony-forming units.

Table 1

Range of initial minimal inhibitory concentrations (MICs) (mg/L), spontaneous mutation frequency (smf) towards fluoroquinolone resistance (4× initial MIC) and selection of high-level meticillin resistance (128 mg/L oxacillin) in the presence of 0.5× the minimal inhibitory concentrations of the study drugs

Phenotype	Parameter	Ciprofloxacin	Levofloxacin	Moxifloxacin
MSSA (<i>n</i> = 24)	MIC	1–16	0.5–16	0.03–1
	smf	2.0×10^{-8} to 2.3×10^{-10}	3.6×10^{-8} to 8.8×10^{-9}	1.0×10^{-9} to $\leq 10^{-11}$
	Resistance selection	0	0	0
HA-MRSA (<i>n</i> = 24)	MIC	4 to >64	4–64	0.12–32
	smf	2.2×10^{-8} to 2.8×10^{-9}	5.2×10^{-8} to 5.0×10^{-9}	1.0×10^{-9} to $\leq 10^{-11}$
	Resistance selection	1.1×10^{-3} to 3.3×10^{-5}	2.2×10^{-4} to 7.1×10^{-5}	3.2×10^{-5} to 4.1×10^{-6}
CA-MRSA (<i>n</i> = 6)	MIC	1 to >64	0.5–64	0.03–16
	smf	2.1×10^{-8} to 3.2×10^{-9}	4.8×10^{-8} to 6.2×10^{-9}	2.2×10^{-9} to $\leq 10^{-11}$
	Resistance selection	4.6×10^{-3} to 6.8×10^{-5}	5.2×10^{-4} to 8.6×10^{-5}	N/D
FQ ^r MRSA (<i>n</i> = 4) ^a	MIC	8 to >64	8 to >64	2–16
	smf	6.4×10^{-7} to 8.4×10^{-8}	2.8×10^{-7} to 5.6×10^{-8}	6.7×10^{-8} to $\leq 10^{-11}$
	Resistance selection	nd	nd	nd

MSSA, methicillin-susceptible *Staphylococcus aureus*; MRSA, methicillin-resistant *S. aureus*; HA, healthcare-associated; CA, community-acquired; FQ^r, fluoroquinolone-resistant; N/D, not done; nd, not done.

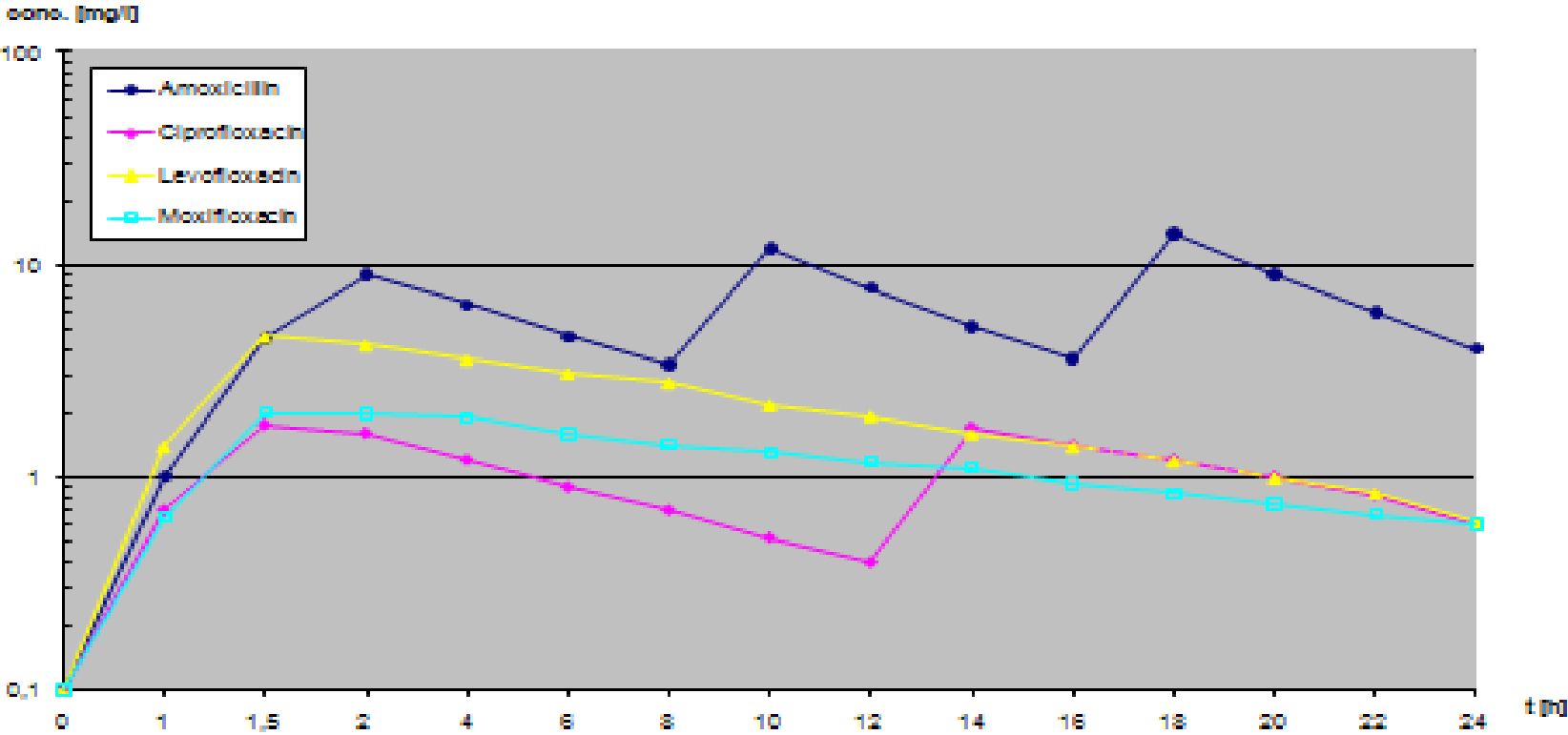
^a FQ^r MRSA strains were generated in the laboratory by serial passage until the initial MIC has been increased eight-fold.

Table 2

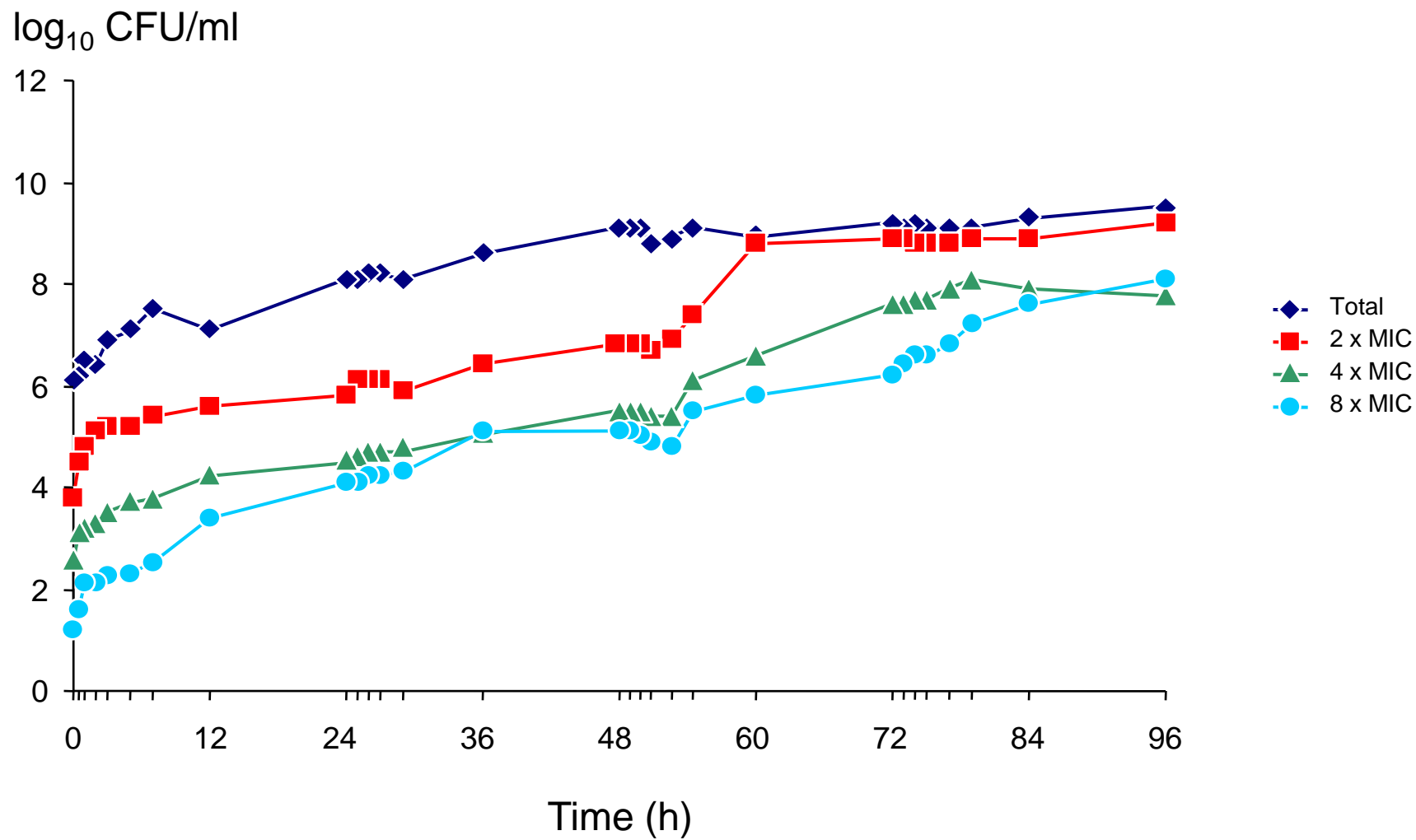
Selection of highly meticillin-resistant (128 mg/L oxacillin) subclones of the heteroresistant meticillin-resistant *Staphylococcus aureus* (MRSA) strain P8 by constant fluoroquinolone concentrations. The parent strain P8, as well as the laboratory-generated mutants with 2×, 4× and 8× elevated fluoroquinolone minimal inhibitory concentrations (MICs), were studied

Fluoroquinolone Concentration (mg/L)	Ciprofloxacin				Levofloxacin				Moxifloxacin			
	Parent	2×	4×	8×	Parent	2×	4×	8×	Parent	2×	4×	8×
		MIC	MIC	MIC		MIC	MIC	MIC		MIC	MIC	MIC
1	5.4 × 10 ⁻⁷	4.6 × 10 ⁻⁶	3.2 × 10 ⁻⁵	2.2 × 10 ⁻⁴	3.6 × 10 ⁻⁹	4.8 × 10 ⁻⁸	7.2 × 10 ⁻⁶	5.8 × 10 ⁻⁵	0	0	0	4.6 × 10 ⁻⁶
5	3.2 × 10 ⁻⁹	5.1 × 10 ⁻⁷	6.2 × 10 ⁻⁶	4.8 × 10 ⁻⁴	0	0	3.2 × 10 ⁻⁸	8.6 × 10 ⁻⁷	0	0	0	0
10	0	1.6 × 10 ⁻⁹	2.8 × 10 ⁻⁷	5.6 × 10 ⁻⁵	0	0	1.2 × 10 ⁻⁹	2.6 × 10 ⁻⁸	0	0	0	0
50	0	0	8.9 × 10 ⁻⁸	7.2 × 10 ⁻⁷	0	0	0	5.6 × 10 ⁻⁹	0	0	0	0
100	0	0	0	1.8 × 10 ⁻⁸	0	0	0	0	0	0	0	0

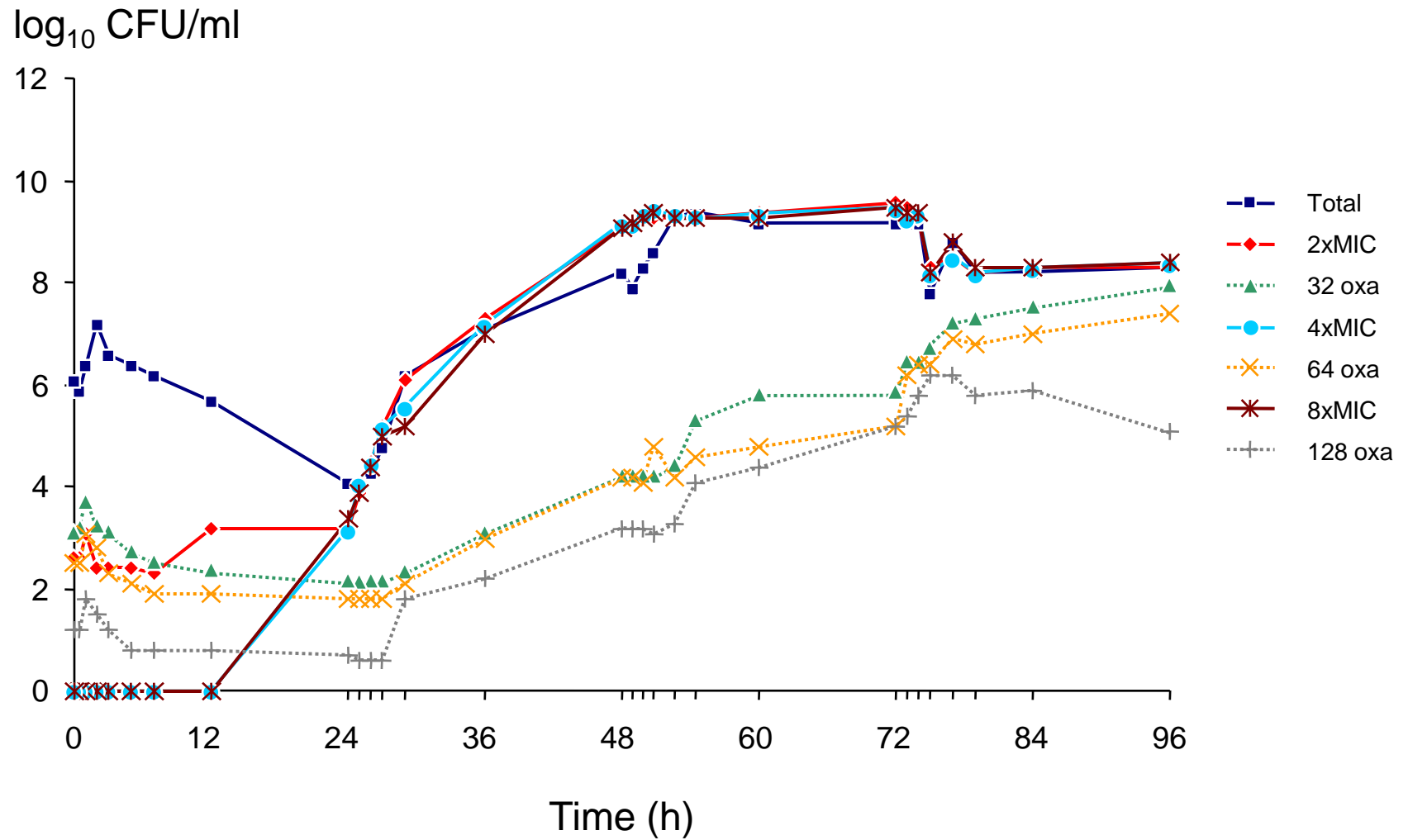
Measured drug concentrations (mg/l) representing the free fraction of amoxicillin 500mg tid, ciprofloxacin 500mg bid, levofloxacin 500 mg qd, and moxifloxacin 400mg qd



Drug Co-Amoxicillin clav. Strain P8+

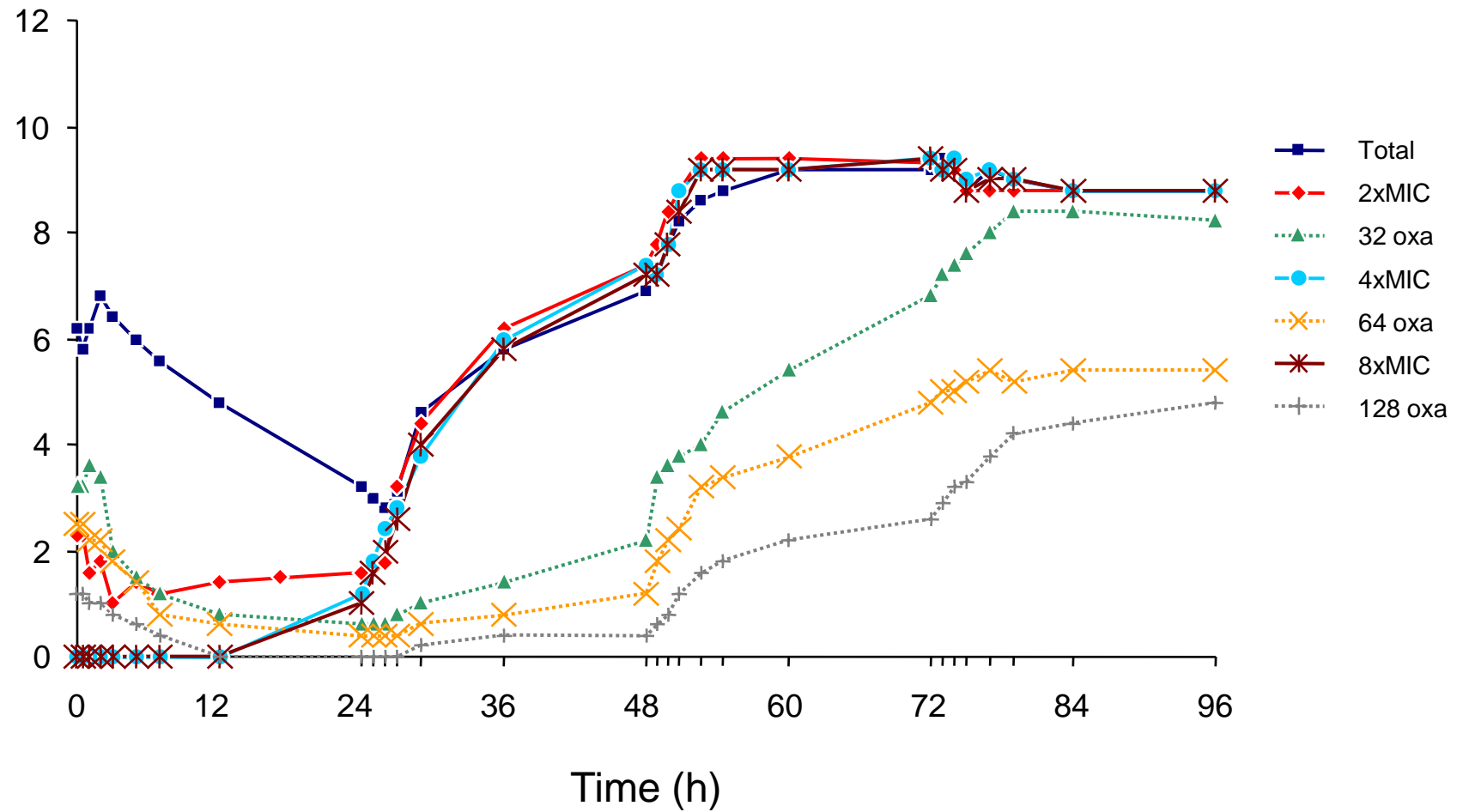


Drug Ciprofloxacin Strain P8+



Drug Levofloxacin Strain P8+

\log_{10} CFU/ml



Drug Moxifloxacin Strain P8+

