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Growth state at drug exposure highly influences the pharmacodynamics of linezolid, levofloxacin, meropenem and vancomycin in time-kill studies with *Staphylococcus aureus*

S. G. Wicha, E. Goebgen, J. Kuss, C. Kloft

Freie Universität Berlin

Department of Clinical Pharmacy and Biochemistry, Institute of Pharmacy, Freie Universitaet Berlin, Germany

Background and Objectives

- Observed **antibacterial effects *in vitro*** are known to be affected by **experimental conditions**.
- Growth medium composition or inoculum size have been identified earlier (e.g. described in Lorian et al. [1]) as influencing factors.
- **Published time-kill curve studies vary** largely with respect to the **pre-incubation time** (0-2 h) before addition of the antibiotic to the bacteria. Consequently, bacteria might either be resting (i.e. in **lag-phase**) or exponentially growing (i.e. in **log-phase**) at drug exposure.
- Hence, we aimed to assess
  (i) the required **time period** to attain the **log-phase** of bacterial growth and
  (ii) the **influence** of the **growth state** at drug exposure on the ***in vitro* pharmacodynamics** of antibiotics acting at different targets, i.e. protein synthesis: linezolid (LZD), DNA: levofloxacin (LEV) and cell wall: meropenem (MER) and vancomycin (VAN) against *S. aureus* (MSSA) as model organism.

Methods

(i) Determination of the lag-time of *S. aureus*

- Lag-time until exponential growth was determined in cation-adjusted Mueller-Hinton broth (MHB, Oxoid, Wesel, Germany) with *S. aureus* ATCC 29213 and two clinical isolates of MSSA. For this purpose, an inoculum of 10^6 CFU/mL was generated with the direct colony suspension method [2] in 10 mL of MHB. Samples were taken every 20 min, and bacteria were quantified using an adapted droplet plate assay [1].
- Lag-time t_{lag} to exponential growth was estimated by non-linear regression analysis using a biphasic exponential growth model describing the number of bacteria N over time with the exponential growth rates k_{lag} and k_{log} and the inoculum $N_{t=0}$ as initial condition IC:

$$\frac{dN}{dt} = k_{growth} \times N \quad IC\colon N_{t=0} \quad \text{with } k_{growth} = k_{lag} \text{ for } t \leq t_{lag}$$
$$\text{and } k_{growth} = k_{log} \text{ for } t > t_{lag}$$

(ii) Influence of the growth state at drug exposure on the antibacterial effect

- For lag-phase experiments, *S. aureus* colonies were sampled from an overnight subculture, suspended in MHB and directly exposed to static antibiotic concentrations of 0x up to 64x MIC (direct colony suspension method [2]).
- For log-phase experiments, *S. aureus* was allowed to grow 2 h before addition of the drug. At time of drug addition, both scenarios had reached an inoculum of 10^6 CFU/mL to avoid other influencing factors, e.g. a reduced effect at higher inoculums.
- Time-kill curve studies were performed with dense sampling (n>8) over 24 h in n≥2 replicates on different days.

Results

(i) Determination of the lag-time of *S. aureus*

Lag-time to exponential growth was between 86 and 102 min. for all investigated strains in the utilised MHB (Fig. 1, Tab. 1).

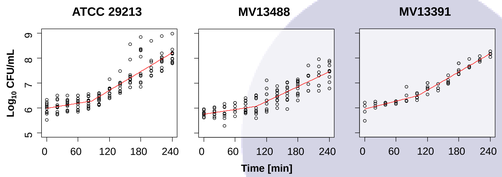

Fig. 1: Biphasic growth after inoculation with two exponential growth rates; intersection indicates t_{lag} for three *S. aureus* strains.

Tab. 1: Lag-times to attain exponential growth of three *S. aureus* strains

S. aureus strain	Lag-time [min] ($CI_{95\%}$)
ATCC 29213	86 (67-105)
MV 13488	99 (95-105)
MV 13391	102 (90-114)

(ii) Influence of the growth state at drug exposure on the antibacterial effect

Linezolid is more active against log-phase *S. aureus* (Fig. 2):

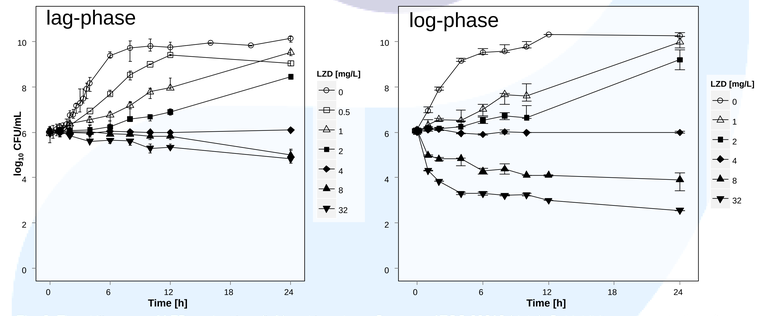

Fig. 2: Time-kill curves of LZD against lag- (left) and log-phase *S. aureus* ATCC 29213 (right). Subinhibitory concentrations (open symbols); inhibitory concentrations (filled symbols); Median (point estimate) and range (error bar) of n=2-4.

Meropenem displays a less marked paradoxical effect [3] at higher concentrations ≥2 mg/L against log-phase *S. aureus* (Fig. 3):

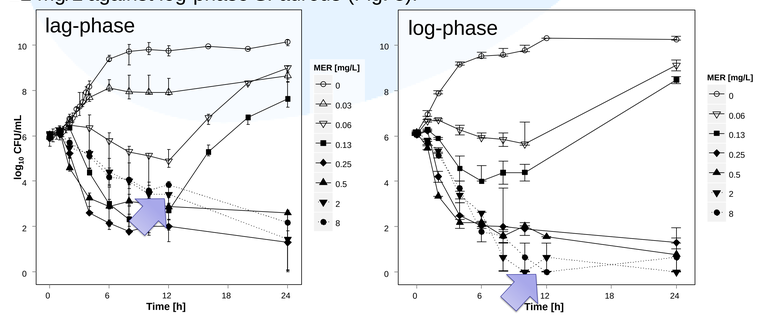

Fig. 3: Time-kill curves of MER against lag- (left) and log-phase *S. aureus* ATCC 29213 (right). Further legend details, cf. Fig. 2.

Results (cont'd.)

Levofloxacin displays slightly faster killing against log-phase *S. aureus* (Fig. 4):

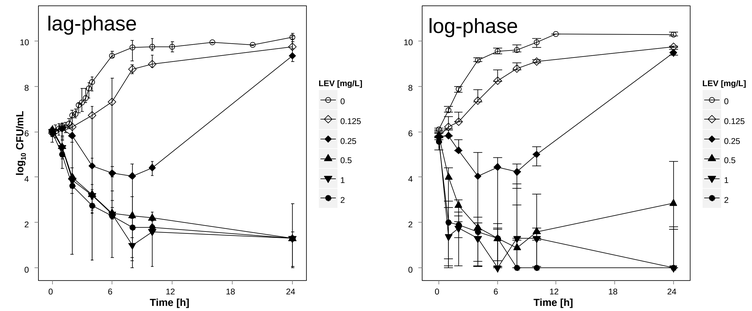

Fig. 4: Time-kill curves of LEV against lag- (left) and log-phase *S. aureus* ATCC 29213 (right). Further legend details, cf. Fig. 2.

Vancomycin exhibits substantially intensified regrowth in log-phase *S. aureus* (Fig. 5):

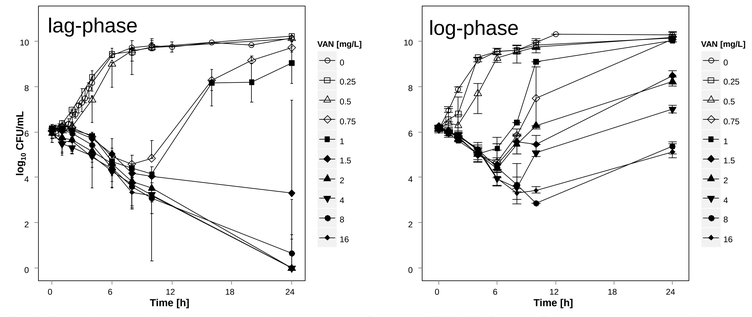

Fig. 5: Time-kill curves of LEV against lag- (left) and log-phase *S. aureus* ATCC 29213 (right). Further legend details, cf. Fig. 2.

Conclusions

- **Lag-time** to exponential growth was ca. **1.5 h** and consistent across **three MSSA strains**.
- The **antibacterial effects** of LZD, MER and VAN were **highly dependent** on the bacterial growth state at drug exposure, whilst the effect of LEV was least affected.
- The consequences ranged from differences in the maximum effect (LZD, MER, LEV) to alteration of regrowth (VAN) and **did not follow a uniform pattern**.
- **Strict standardisation** and **reporting** of the experimental conditions seem **crucial** to enable **reproducible and comparable time-kill curve studies** and are currently often neglected when these are performed.

References

[1] V. Lorian. Antibiotics in Laboratory Medicine. Lippincott Williams & Wilkins, 5th ed. (2005).
[2] Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard-Seventh Edition M7-A9. (2012).
[3] H. Eagle, A. Musselman. *J. Exp. Med.*, 35: 220–5 (1948).

For additional information, please contact
Sebastian G. Wicha
sebastian.wicha@fu-berlin.de

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