Drug Monitoring und Toxicology **Edited by: W. Steimer**

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Personalized antibiotic therapy – a rapid high performance liquid chromatography–tandem mass spectrometry method for the quantitation of eight antibiotics and voriconazole for patients in the intensive care unit

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Abstract

Objectives: For a long time, the therapeutic drug monitoring of anti-infectives (ATDM) was recommended only to avoid the toxic side effects of overdosing. During the last decade, however, this attitude has undergone a significant change. Insufficient antibiotic therapy may promote the occurrence of drug resistance; therefore, the "one-dose-fits-all" principle can no longer be classified as up to date. Patients in intensive care units (ICU), in particular, can benefit from individualized antibiotic therapies.

Methods: Presented here is a rapid and sufficient LC-MS/MS based assay for the analysis of eight antibiotics (ampicillin, cefepime, cefotaxime, ceftazidime, cefuroxime, linezolid, meropenem, and piperacillin) applicated by continuous infusion and voriconazole. In addition a dose adjustment procedure for individualized antibiotic therapy has been established.

Results: The suggested dose adjustments following the initial dosing of 121 patient samples from ICUs, were evaluated over a period of three months. Only a minor percentage of the serum levels were found to be within the target range while overdosing was often observed for β-lactam antibiotics, and linezolid tended to be often underused. The results demonstrate an appreciable potential for β-lactam savings while enabling optimal therapy.

Conclusions: The presented monitoring method provides high specificity and is very robust against various interferences. A fast and straightforward method, the developed routine ensures rapid turnaround time. Its application has been well received by participating ICUs and has led to an expanding number of hospital wards participating in ATDM.

Keywords: anti-infective agents; drug monitoring; intensive care units; liquid chromatography; mass spectrometry.

Introduction

The benefit of therapeutic drug monitoring of anti-infectives (ATDM), particularly antibiotics, has long been controversially discussed. For a long time, the application of ATDM was only recommended for a few antibiotics (e. g. aminoglycosides) in order to avoid the toxic side effects of overdosing [1]. However, during the last decade, this attitude has undergone a significant change [2] due mainly to the widely increasing occurrences of antibiotic resistance [3–6], and the limited development of new antimicrobial agents [7]. As insufficient antibiotic therapy may promote the occurrence of drug resistance [8], the "one-dose-fits-all" principle, especially for critically ill patients, is no longer considered to be up to date. Altered pharmacokinetics and pharmacodynamics (PK/PD) through one or more dysfunctional organ system, sepsis, dialysis, hypoalbuminemia or extensive burns may bear a high risk of under- or overdosing, thus making it difficult for clinicians to find the right dosage for individual patients [2, 9, 10]. A recent study stated that a substantial proportion of intensive care unit (ICU) patients

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with insufficiently low antibiotic levels, have a higher mortality rate and suffer from greater infection severity [11]. Therefore, when pharmacokinetics is difficult to predict [12], ATDM is commonly used. Meanwhile, dose optimization by ATDM is recommended by the DGI S3-guideline for the antibiotic stewardship (ABS) [13] and Surviving Sepsis Guidelines (SSG) [14].

In Germany, the most frequently prescribed anti-infectives in ICUs are β-lactam antibiotics [15]. Additionally, linezolid as a last resort antibiotic and voriconazole as an antimycotic against invasive aspergillosis are important therapeutic agents. While commonly accepted trough-level ranges exist for voriconazole [16], correlations between serum concentration and therapeutic efficiency of antibiotics are more complex and depend on several factors such as application form, dosing interval, co-medication, and focus of infection. For an intermitting application of β-lactams, the time above minimum inhibitory concentration (T>MIC) is one parameter that correlates best with clinical efficacy. Whereas T>MIC should exceed 50% for penicillin, 60–70% is recommended for cephalosporins, and 40% for carbapenems [17]. In order to fit the time-dependent concentration curves, accurate measurements of at least 2 (better 3–5) time points are required. A continuous infusion is simpler to handle, where serum level should be the four to five-fold of the MIC [18–21] while sampling is possible at any point of time in steady-state.

ATDM is often requested for just a small number of patients, but demand short turn-around-times (TAT). Fully automated immunoassays would therefore be the first method of choice, allowing integration into routine sample processing and at the same time providing quick TATs. Unfortunately, however, only a limited number of such assays are currently commercially available (e.g. amikacin, vancomycin, tobramycin, gentamycin). Several (U)HPLC-UV methods for antibiotic determination have also been described in the literature [22, 23]. Such methods offer a cost-efficient approach to ATDM as the required instrumentation is widely available, but may determine a few substances only [24–28]. The multi-methods as mentioned above, among them a commercial kit method [29], often exhibit the disadvantages of long run-times, possible interferences, and are limited in the number of analytes they measure [30–33]. The utilization of LC-MS/MS has generally grown in prominence during the past years, leading to increasing interest in the use for ATDM due to its specific, sensitive and fast measurements. A growing number of articles are being published, documenting the use of multi-analyte methods for antimicrobials [34–38]. Interestingly, investigations into analysing the benefit of all ATDM substances and the calculation of subsequent dose adjustment are sparse.

This paper will focus on the development of a rational, rapid and sufficient ATDM assay for the analysis of eight antibiotics, ampicillin (AMP), cefepime (CEF), cefotaxime (CFO), ceftazidime (CFT), cefuroxime (CFU), linezolid (LIN), meropenem (MER), and piperacillin (PIP) applicated by continuous infusion and voriconazole (VOR), as well as a procedure for dose adjustment for individualized antibiotic therapy. Therefore a previously published method [37] was adapted to our particular needs. Furthermore, a critical view on the experiences from several ICUs over a three month ATDM test period will also be given.

Materials and methods

Instrumentation

Chromatographic analyses were performed using a Nexera XR series high-performance liquid chromatography system (Shimadzu, Jena, Germany) with two pumps, vacuum degassers, columns oven and a CTC-xt autosampler (PAL, Zwingen, Switzerland) coupled to a Sciex 5500 QTRAP AMCR triple-quadrupole mass spectrometer equipped with electrospray (ESI) source (Sciex, Darmstadt, Germany). The Peltier sample trays and oven temperature were set to 8 and 50 °C, respectively. For the chromatographic separation, a reverse phase YMC-Triart PFP S-3um/12 mm column (50 × 2.0 mm I.D.; YMC, Dinslaken, Germany) with a nylon prefilter (Recipe, Germany) was used. Mobile phases A (Water + 0.1% formic acid, MPA) and B (methanol, MPB) were delivered through a total flow rate of 0.65 mL/min in a gradient elution mode. Starting at 4% of MPB for 0.15 min, a linear increase to 60% at 1.7 min followed. From 2.3 to 3.0 min, another increase from 60 to 90% MPB was applied and maintained at 90% up to a run time of 3.8 min. The column was then re-equilibrated to the initial conditions up to a total run time of 4 min. Eluting compounds were detected in multiple reactions monitoring (MRM) with switching polarity electrospray ionization at an ion spray source voltage (IS) of 5500 V in positive mode and −4500 V in negative mode, respectively. Nebulizer gas (GAS 1) and turbo gas (GAS 2) were set at 50 psi, curtain gas (CUR) to 35 psi, the collision gas (CAD) to 8 psi, and desolvation temperature (TEM) to 400 °C. Detailed MRM-transitions (Q1 and Q3), declustering potentials (DP), collision energies (CE), cell exit potentials (CXP), entering potentials (EP) and dwell times of the analytes are listed in Table 1. For data acquisition and processing Analyst software (1.7.0) and Multi Quant Software (3.0.2) were used (Sciex, Darmstadt, Germany).

Standards and reagents

Water, methanol, acetonitrile, and formic acid (all LC-MS grade) were supplied by Diagonal (Münster, Germany). MER-$^{2}H_6$, PIP-$^{2}H_5$, LIN-$^{2}H_3$, VOR-$^{2}H_3$, and CFO-$^{13}C^{2}H_3$ were purchased from ALSACHIM (Illkirch Graffenstaden, France), and oxacillin (OXA) from Sigma-Aldrich (Hamburg, Germany). Antibiotic infusion solutions (Ampicillin Ratiopharm, Cefepim Rotexmedica, Cefotaxim Fresenius, Ceftazidim Kabi, Cefuroxim Fresenius, Meropenem Kabi, Piperacillin Fresenius, Voriconazole Ratiopharm, Zyvoxid Pfizer) were obtained through the Klinikum Chemnitz dispensary. Fresh frozen plasma was obtained

Table 1: Parameters of the MS/MS method.

Q1, *m/z*	Q3, (*m/z*)	ID	DP, V	EP, V	CE, V	CXP, V	Dwell, ms
350.0	91.0	AMP 1	116	10	83	16	10
350.0	160.0	AMP 2	116	10	19	10	5
241.0	126.0	CEF 1	51	10	33	12	10
241.0	86.0	CEF 2	51	10	17	12	5
455.9	395.9	CFO 1	1	10	13	20	20
455.9	323.8	CFO 2	1	10	19	42	10
459.9	399.9	CFO-$^{13}C^2H_3$	1	10	13	20	5
273.9	125.8	CFT 1	21	10	31	12	10
273.9	111.8	CFT 2	21	10	37	16	5
338.0	296.1	LIN 1	196	10	29	18	10
338.0	235.1	LIN 2	196	10	31	22	10
341.0	297.1	LIN-$^2H_3$	196	10	29	18	5
384.0	141.0	MER 1	16	10	23	10	20
384.0	68.0	MER 2	16	10	89	14	10
390.0	160.0	MER-$^2H_6$	16	10	23	10	5
518.1	147.1	PIP 1	101	10	15	10	20
518.1	160.0	PIP 2	101	10	85	8	10
523.1	115.0	PIP-$^2H_5$	101	10	15	10	5
350.0	160.0	VOR 1	141	10	23	6	10
350.0	281.1	VOR 2	141	10	27	16	5
353.0	224.1	VOR-$^2H_3$	141	10	23	6	10
402.2	284.0	OXA (pos.)	166	10	17	10	20
423.0	207.0	CFU 1	−80	−10	−10	−22	10
423.0	318.1	CFU 2	−80	−10	−10	−12	5
400.0	259.0	OXA (neg.)	−55	−10	−10	−10	20

AMP, ampicillin; CEF, cefepime; CFO, cefotaxime; CFT, ceftazidime; CFU, cefuroxime; LIN, linezolid; MER, meropenem; OXA, oxacillin; PIP, piperacillin; VOR, voriconazole.

from the laboratory blood bank and tested for interferences before further use.

Preparation of stock solutions, standards, quality control samples (QCs), and internal standard solutions

For the preparation of stock solutions MER-$^2H_6$, PIP-$^2H_5$, LIN-$^2H_3$, CFO-$^{13}C^2H_3$, and VOR-$^2H_3$ each of the compounds (1 mg) were dissolved in methanol (1 mL), while OXA (15 mg) was dissolved in 5 mL of acetonitrile/water (4 + 1). The internal standard solution was prepared by adding the stock solutions of LIN-$^2H_3$, VOR-$^2H_3$, and OXA (25 µL each) as well as MER-$^2H_6$, PIP-$^2H_5$, CFO-$^{13}C^2H_3$ (100 µL each) to a mixture of acetonitrile (5 mL) and methanol (5 mL). The solution was stored at −20 °C until further use. Antibiotics stock solutions were prepared by diluting antibiotic infusion solutions of the individual substances in water. Blank plasma aliquots were then spiked with 10% volume of respective antibiotic stock solutions. The preparation of matrix calibrators and QC was done independently and with different vials of substances. The accuracy of the prepared standards and QCs was checked against commercially available antibiotic serum QCs (Chromsystems, Germany, Munich) which are traceable to gravimetrically prepared and certified materials. A deviation of less than 5% was found for the prepared standards and QCs, which is within the method coefficient of variation (CV).

Sample preparation

Patient blood samples were stored and transported under cooled conditions until arriving in the laboratory within 3 h. After centrifugation, the serum samples were analysed immediately or stored at −20 °C. Fifty micro litre of the prepared internal standard solution was added to 25 µL serum. The samples were vortexed for 30 s and centrifuged (10,000 *g*) at ambient temperature for 5 min to remove proteins. Twenty µL of the supernatant was transferred into a glass vial, diluted with 500 µL of a water/methanol (4 + 1) solution, and thoroughly mixed before injection (2 µL) for chromatographic analysis.

Method validation

For daily measurements, a four-level-calibration with an additional blank sample was performed. Calibrator concentrations ranged from approximately 50% of the lowest expected MIC to the 20-fold concentration of the highest expected MIC (Table 2) and was tested for linearity in this range. Calibration curves were calculated by linear regression through zero from the ratio of respective peak areas and internal standard areas. Overcoming quality control constraints meant that the concentrations had to be within a 20% range of their target values. Limits of blank (LOB), limits of detection (LOD), and LLOQ (lower limit of quantitation) were calculated according to the literature [39]. The lowest limits of quantification for ATDM were assigned to 50% of the lowest standard concentration. These could have been analytically lower, but further quantification would be clinical irrelevant (Table 2).

Calculations of intra-assay precision and accuracy were performed through 21 replicate measurements of the two quality control samples. Inter-assay precision and accuracy were calculated following the measurements of both QCs a total of 20 times. Calibration was performed before each run with a maximum of four runs within 24 h. Accuracy (in %) was calculated as *100 × concentration observed/nominal concentration* while precision (in %) was described as the relative standard deviation (RSD) of the measured values (Table 3). Selectivity and specificity of the selected MRM-transitions were tested by measuring serum samples of 140 possibly interfering drugs

Table 2: LODs, LLOQ, and reported ranges for antibiotic drug monitoring of the investigated anti-infectives.

	LOB, mg/L	LOD, mg/L	LLOQ , mg/L	Reported range/linearity, mg/L
AMP	0.14	0.20	0.49	2.5–180
CEF	0.20	0.30	0.83	2.5–290
CFO	0.15	0.24	0.67	1.0–60
CFT	0.29	0.41	1.03	2.5–360
LIN	0.13	0.18	0.45	0.5–75
MER	0.08	0.12	0.32	1.0–90
PIP	0.25	0.36	0.92	2.5–290
VOR	0.007	0.010	0.024	0.1–8.0
CFU	0.14	0.20	0.54	2.5–180

AMP, ampicillin; CEF, cefepime; CFO, cefotaxime; CFT, ceftazidime; CFU, cefuroxime; LIN, linezolid; MER, meropenem; OXA, oxacillin; PIP, piperacillin; VOR, voriconazole.

Table 3: Intra- and inter-assay precision and accuracy of the anti-infectives monitored.

	Conc., mg/L	Precision intra-assay	Accuracy intra-assay	Precision inter-assay	Accuracy inter-assay
AMP	12.5	4.5%	100.7%	3.7%	100.4%
CEF	16.0	8.3%	101.9%	2.9%	98.8%
CFO	5.0	4.0%	96.8%	4.9%	97.2%
CFT	20.0	5.6%	106.2%	4.8%	99.6%
LIN	5.0	2.8%	99.9%	3.2%	101.2%
MER	7.50	4.8%	104.2%	3.2%	100.5%
PIP	25.0	5.4%	95.6%	2.9%	98.4%
VOR	0.50	4.0%	99.8%	2.8%	99.4%
CFU	12.5	3.2%	106.7%	5.0%	98.9%

AMP, ampicillin; CEF, cefepime; CFO, cefotaxime; CFT, ceftazidime; CFU, cefuroxime; LIN, linezolid; MER, meropenem; OXA, oxacillin; PIP, piperacillin; VOR, voriconazole.

and metabolites (antibiotics, antimycotics, antidepressants, antiepileptic drugs, benzodiazepines, neuroleptics, immunosuppressants, pain drugs, stimulants, and drugs of abuse), at pharmacologically relevant concentrations. Blank samples were injected after the analysis of the highest calibrator. These experiments were repeated four times for five days. A response higher than 5% of LLOQ was not observed for any of the compounds.

Sample stability

According to the literature MER, PIP, and AMP are the least stable among the investigated substances. Blood samples of patients treated with MER, PIP, and AMP were evaluated for sample stability, benchtop handling and long term storage. Longer stability was assumed for all other compounds. Whole blood samples from six patients were collected from the ICU in serum Monovette® tubes (Sarstedt, Nümbrecht, Germany) and stored at 2–8 °C for 10, 30, 60, 120 and 180 min until sample preparation. A sample was considered to be stable if the concentration decline between the tested conditions was less than 10%.

Analysis of patient samples and PK/PD dose adjustment

Serum samples were taken from ICU patients after approximately 24 h of antibiotic therapy (loading dose followed by continuous infusion (Table 4). After this time a steady-state of antibiotic-serum-concentration could be assumed. According to the focus of infection, PK/PD dose adjustments were aimed at reaching four times or eight times (for deep-seated infections) of the MIC of the suspected or identified pathogen [40]. Dose adjustments were made based on the equation *last daily dose* × *target MIC/measured serum concentration*. In addition, the dose adjustment took into account parameters such as renal function including renal replacement therapy and serum markers for bacterial infection (e.g. procalcitonin, C-reactive protein). If, for example, dialysis was planned for a patient in a time-related context with a dose reduction, the antibiotic dose was not reduced despite the currently existing renal insufficiency. The dose required to achieve the corresponding target MIC for patients with dialysis was calculated according to the internet-based software "CADDy" [41].

Results

Method development

The eight investigated β-lactam antibiotics revealed pH--dependent retention behaviour. At neutral conditions, poor retention was only observed for MER, CFO, and AMP, which made chromatographic separation impossible. The addition of 0.1% formic acid to MPA (pH 2.7) improved both retention and chromatographic separation. In the literature, PFP phases are often recommended for the chromatographic separation of antibiotics [37]. Thus a column (50 × 2 mm ID, 2.6 µm) containing a core–shell material was tested which showed good separation performance but a broad peak for CEF and co-elution of CFT and MER. Under the same conditions, a fully porous PFP phase (50 × 2 mm ID, 3 µm) revealed no peak broadening or tailing, and chromatographic separation of all compounds. Mass spectrometer and ionization parameters were optimized for the maximum response. To enable high throughput measurements for all substances in clinically relevant concentration ranges, the most intensive mass transitions of

Table 4: Serum level evaluation after initial dose.

	CFT	MER	PIP	LIN
Daily dose (continuous infusion) following infection focus	6 g/d	3 g/d	13.5 g/d	1.2 g/d
Loading dose	2 g	1 g	4.5 g	0.6 g
<50% of target	0	0	0	10
50–80% of target	0	4	4	9
80–120% (target range)	1	8	10	4
120–300% of target	4	25	19	2
>300% of target	5	7	9	0
Total samples	10	44	42	25
Observed concentrations, mg/L	29.3–138	5.1–45.0	17.3–262	1.4–15.3
Median concentration, mg/L	59.4	14.5	65.4	5.4
MIC ranges, mg/L	4–64	1–16	16–32	4–10
MIC median, mg/L	32	8	32	8.25

CFT, ceftazidime; LIN, linezolid; MER, meropenem; PIP, piperacillin.

AMP (350.0→106.0), LIN (518.1→143.1), and PIP (338.0→296.1) were replaced by weaker transitions to avoid detector saturation. All compounds were ionized in positive mode except for CFU and OXA. The latter of which was used as an internal standard compound for both positive and negative mode. For all 140 substances tested for possible interferences, no response was found to be higher than 10% of LLOQ for quantifier and qualifier MRM-transition, or 5% of the internal standards within a ±25% window of the expected retention time. Figure 1 shows an MRM chromatogram of a QC sample containing all anti-infectives and internal standards. Isotope labelled substrates and OXA were tested as internal standard substances.

Only the quantifier mass transitions are shown. During the measurement a polarity switch (ESI+/ESI–) was made. 1: cefepime (CEF), 2: ceftazidime (CFT), 3: meropenem (MER), 4: cefuroxime (CFU), 5: cefotaxime (CFO), 6: ampicillin (AMP), 7: linezolid (LIN), 8: piperacillin (PIP), 9: oxacillin (OXA), 10: voriconazole (VOR).

Surprisingly, the comparison of oxacillin and the specific isotope labelled compounds as internals standards showed nearly no differences. Just for MER and CFO a systematic deviation of approx. 10% was observed.

Sample stability

Sample stability at 2–8 °C was verified for up to 3 h. It was shown to be sufficient for in-house-monitoring, as analyses were always performed at an assigned time. Furthermore, sample drawing, as well as transportation, could be organized within that stability period in our setting. Patient blood samples were stored and transported under cooled conditions until analysis and immediately prepared upon arrival at the laboratory. Following sample preparation, no loss of concentration was observed after 8 h at ambient conditions (25 °C) or after 24 h at 2–8 °C. Samples frozen at –80 °C for long term storage showed to be stable for at least three months.

Dose adjustments

The suggested dose adjustments after initial dosing were evaluated over three months, with a total of 121 samples from ICU-patients (Table 4, Figure 2). Due to the small number of samples received for some analytes, an evaluation was only justifiable for CFT (10 samples), MER (44 samples), PIP (42 samples), and LIN (25 samples). For MER the measured serum concentrations ranged from 5.1 to 45.0 mg/L (median 14.5 mg/L) while the MICs varied from 1.0 to 16 mg/L (median 8 mg/L. Higher serums levels than aspired were observed in 32 of 44 samples, while only four samples showed moderate underdosing of 50–80% of the desired target value. Overdosage, with levels more than three times higher than aspired values, was found in seven patients. CFT and PIP showed similar results. Moderate to

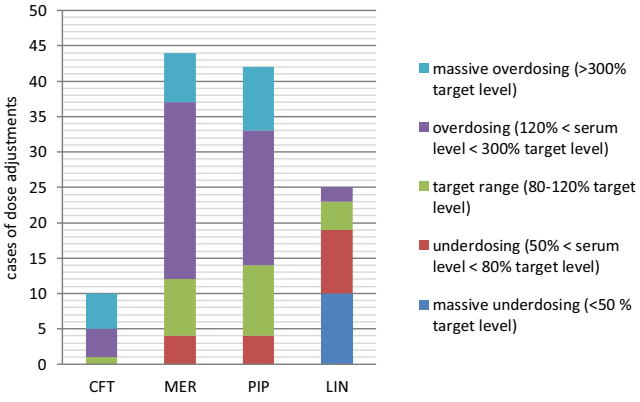

Figure 2: Serum level evaluation and suggested dose adjustments after initial dosing.

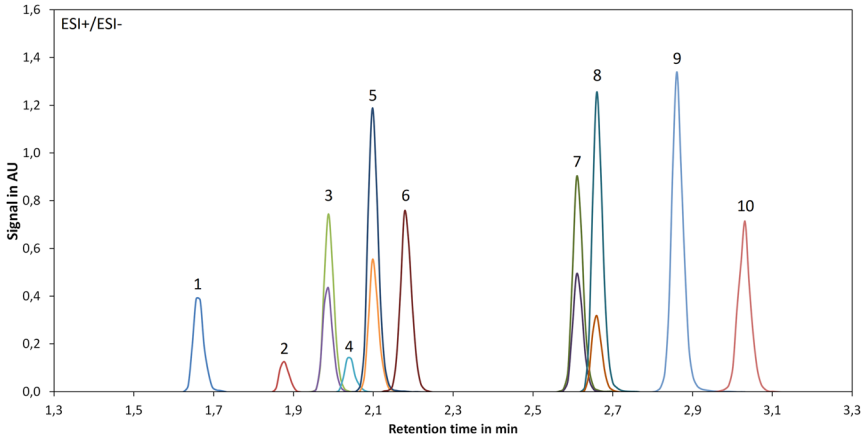

Figure 1: Chromatogram of a QC sample.

high overdosing was observed in 9 of 10 samples for CFT and 28 of 42 samples for PIP, while moderate underdosing was only seen in four sample cases each. Severe overdosing of 1,000–2,000% for MER and CFT was observed on five occasions. Underdosing was frequently observed in patients (19 of 25) treated with LIN (10 of them showing a serum concentration of less than 50% of the desired target value). Overdosage of LIN therapy was detected in only two cases (131 and 185%).

Especially β-lactam antibiotics tend to be overdosed, showing the saving potential in clinical use. Linezolid turned out to be under dosed often, which may result in the occurrence of resistances. Ceftazidime (CFT), linezolid (LIN), meropenem (MER), piperacillin (PIP).

Discussion

The presented method allows the analysis of nine anti-infectives usually prescribed in ICUs within a runtime of only 4 min. The applied LC-MS/MS technique provides high specificity, is very robust against various interferences, and also provides adequate sensitivity. In addition, it also grants a wide linear measuring range which is suitable for routine clinical measurements, without the need for further dilution at high levels (Table 2). Sample preparation is based on simple protein precipitation which allows for the determination of total serum concentrations, and for the preparation of large sample numbers in less than 15 min.

For a personalized antibiotic therapy, PK/PD-calculations and dose adjustments were performed and communicated as a simple report with a proposed adjustment of infusion rates for the respective antibiotics. The strict time and organizational plan allow for therapeutic TATs of less than 6 h after initial sampling. The simple and fast routine that was developed to ensure a short TAT was well-received by participating ICU and has led to an expanding number of hospital wards participating in ATDM. As reported in other studies [42, 43], we also only found a minor percentage of the serum levels to be in the target range (Table 4).

Severe overdosing of β-lactam antibiotics occurred when sensitive pathogens with low MICs were found in microbiological analysis or in patients with severely impaired renal function who had received an antibiotic standard dose. ATDM allowed massive reductions of the daily doses of, e.g. MER in such cases. In general, overdosing occurs, particularly in β-lactam antibiotic therapies. Due to their low toxicity, this may not be critical, but in some cases, too high concentrations of β-lactam antibiotics can cause severe adverse reactions [44]. To summarize, this demonstrates an appreciable potential for β-lactam savings while enabling optimal therapy. In the case of LIN, underdosing proved to be more problematic for patients. Inadequate treatment can lead to therapeutic failure and resistant pathogen formation. High serum levels are somewhat rare following a standard dose of LIN and instead, too low serum levels are more commonly measured. Therefore in response, a general increase in the initial LIN dosage could be a reasonable approach. As a limitation, it must be mentioned that the results were not evaluated regarding impaired renal function or organ replacement procedures or the clinical cure of the patients. The financial benefit of ATDM is questionable. Even with a reduction in antibiotics consumption, analytical steps and costs must be taken into account and also the fact that daily dosage costs of the investigated β-lactams are low. Nevertheless, ATDM is a versatile tool which can be especially useful for patients in ICUs with altered PK/PD to ensure optimal therapy.

Conclusions

In this study, we demonstrated a robust yet simple high throughput method for the determination of eight antibiotics and VOR in serum. A test phase on ICUs in routine clinical conditions has proven its practical use and has been widely accepted. The evaluation of 121 patient serum samples has shown a high variability. For an ATDM based treatment with the β-lactams CFT, MER, and PIP there appear to be universal savings. Whereas for the drug of last resort, LIN, underdosing seems to be more common than overdosage. Our findings should be further investigated with respect to therapy failure and possible antibiotic resistance formation due to underdosing, or the toxic side effects due at overdosing.

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Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the authors' institutional review board or equivalent committee.

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