Journal of Mass Spectrometry and Advances in the Clinical Lab 34 (2024) 46–54

Contents lists available at ScienceDirect

Journal of Mass Spectrometry and Advances in the Clinical Lab

journal homepage: www.sciencedirect.com/journal/journal-of-mass-spectrometry-and-advances-in-the-clinical-lab

Research Article

Development and clinical utility of an ultra performance liquid chromatography – tandem mass spectrometry assay for monitoring omadacycline and tigecycline in severe bacterial infections

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ARTICLE INFO

Keywords:
Omadacycline
Tigecycline
UPLC-MS/MS
Plasma concentration
Severe infection

ABSTRACT

Objective: We aimed to develop a rapid, simple, and precise ultra performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS) technique for simultaneous measurement of omadacycline (OMA) and tigecycline (TGC) in the bloodstream of individuals suffering from serious bacterial infections.

Methods: All analytes were extracted using a 0.2 % formic acid–water dilution and acetonitrile plasma protein precipitation. The quantification was performed by electrospray ionization-triple quadrupole mass spectrometry with selected reaction monitoring and positive ion mode detection. Tetracycline was used as an internal standard in this experiment, with the mobile phase composed of water (with 0.1 % formic acid) and acetonitrile (using gradient elution) flowing at a rate of 0.35 ml/min, and the column temperature set at 30 °C. Each individual analysis was completed in under 3.5 min.

Results: The method was validated based on FDA recommendations, including the assessment of extraction recovery (92.65–101.72 %) and matrix effects (86.22–91.12 %). The standard curve ranges for both OMA and TGC are 0.025 μg/mL to 2.5 μg/mL. The plasma samples were found to be consistent after undergoing three rounds of freezing and thawing at room temperature for 24 h, being placed in an automated sample injector for 24 h, and then frozen for 45 days. Clinical cases were used to demonstrate the application of the therapeutic drug monitoring (TDM) assay, showing how an analytical test can quickly provide information on antibiotic levels in patients and impact their treatment.

Conclusion: Multiplex UPLC-MS/MS assays for the simultaneous measurement of plasma OMA and TGC concentrations are the ideal choice for clinically TDM applications.

Introduction

Antimicrobial resistance (AMR) is one of the top ten global health threats [1]. The urgent issue of bacterial resistance to antibiotics poses a significant challenge to global public health and the economy. Approximately 495 million deaths in 2019 were attributed to bacterial

Abbreviations: AMR, Antimicrobial resistance; CRRT, continuous renal replacement therapy; ECMO, extracorporeal membrane oxygenation; ESI, Electrospray ionization source; G+, Gram-positive; G-, Gram-negative; ICU, Intensive care unit; IS, internal standards; IS-MF, IS-normalized matrix factor; LLOQ, lower limit of quantification; MDR, multidrug-resistant; MF, Matrix factor; MIC, minimum inhibitory concentration; OMA, omadacycline; PK/PD, pharmacokinetic/pharmacodynamic; QC, quality control; RT, room temperature; RFT, repeatedly frozen and thawed; SRM, Selective reaction monitoring; S/N, signal-to-noise ratio; TDM, therapeutic drug monitoring; TGC, tigecycline; ULOQ, upper limit of quantitation; UPLC-MS/MS, ultra performance liquid chromatography – tandem mass spectrometry.

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https://doi.org/10.1016/j.jmsacl.2024.11.001
Received 12 May 2024; Received in revised form 19 October 2024; Accepted 15 November 2024
Available online 22 November 2024

antibiotic resistance, including 127 million deaths caused by bacterial AMR, according to Murray et al. [2]. The mortality rates surpass those of HIV/AIDS, malaria, and drug-sensitive tuberculosis in individuals without HIV, as well as previous alerts [3–6]. According to the World Bank, as much as 3.8 % of the worldwide gross domestic product could be at risk because of AMR by the year 2050 [7]. Improper choice of medication and inadequate antibiotic dosing (either low dose or short treatment duration) are the primary factors that contribute to resistance [8]. However, monitoring the therapeutic drug concentration of special-grade antibiotics can help adjust the dosage of antibiotics in a timely manner and improve the effectiveness of antibiotics in clinical treatment.

Even with numerous advancements in treatment, the mortality rate from infections in critically ill patients continues to be a significant concern in healthcare. Intensive care units (ICUs) consume 10 times more antibiotics than other wards, attributed to the correspondingly high infection burden [9]. Optimizing antibiotic use is crucial not just for enhancing treatment effectiveness but also for extending the lifespan of current medications by reducing the development of drug resistance [10,11]. In this specific group of patients, inadequate antimicrobial dosing could result in insufficient drug levels, potentially leading to treatment failure or antibiotic resistance [12], while excessive drug levels could increase the risk of toxicity. Recently, there has been a rise in the prevalence of bacterial pathogens that are resistant to multiple drugs, causing a growing number of individuals globally to be impacted by these organisms. This presents a significant danger to human health due to extended hospitalizations and high rates of illness and death [13,14]. Therefore, making full use of the therapeutic drug monitoring (TDM) results for patients with severe bacterial infections by establishing individualized dosage regimens is particularly important.

Tigecycline (TGC), a novel glycylcycline antimicrobial agent, exhibits broad-spectrum antibacterial properties and potent activity against both Gram-positive (G +) and Gram-negative (G-) pathogens, particularly various drug-resistant bacteria like vancomycin-resistant *Enterococcus*, methicillin-resistant *Staphylococcus aureus*, and carbapenem-resistant *Acinetobacter baumannii*. Presently, it is commonly utilized in medical settings to manage patients with multidrug-resistant (MDR) and severe, complex infections [15]. The potential benefits of the association between TGC and TDM have been extensively explored [16]. Furthermore, clinical studies related to pharmacokinetic modeling and simulation have demonstrated the importance of TDM in guiding the treatment of many bacterial infections with TGC [17].

Omadacycline (OMA), a new aminomethylcycline developed by Paratek Pharmaceuticals (Boston, Massachusetts), displays strong antimicrobial properties against various types of bacteria, including G+ and G- aerobic bacteria, anaerobic bacteria, and especially pandrug-resistant pathogens, as well as atypical pathogens [18,19]. Modifications at C7 and C9 in the OMA structure enable it to overcome two of the most common mechanisms of bacterial resistance to tetracycline antibiotics: efflux pumps and ribosome protective proteins. Recent microbiological data suggest that OMA exhibits *in vitro* activity against various resistant pathogens [20].

While many studies have utilized liquid chromatography-mass spectrometry (LC-MS) for measuring antibiotic levels, the majority have concentrated on β-lactam antibiotics, antifungal drugs, and glycopeptide antibacterials. Only a few studies have reported the application of TDM for TGC in critically ill patients. As a newly marketed antibiotic, OMA has limited clinical application experience, and no TDM studies on OMA have been conducted.

Moreover, ICU patients often present various pathological and physiological conditions, such as hypoalbuminemia, continuous renal replacement therapy (CRRT), and extracorporeal membrane oxygenation (ECMO), which lead to significant individual differences in drug exposure. ICU doctors have not consistently achieved the desired therapeutic effect when using OMA and TGC according to the recommended dosages in the instructions. However, with the assistance of TDM, doctors can determine whether antibiotics have reached the inhibitory dose. Consequently, we developed and confirmed an ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) technique and conducted quantitative analysis and therapeutic drug monitoring of TGC and OMA in human plasma to assist in the appropriate administration of antibiotics in severely ill individuals.

Materials and methods

Chemical substances and laboratory materials

Omadacycline tosylate (95.9 % purity, lot number: CPo121470-04-05-01-RS) was purchased from Asymchem Laboratories Co., Ltd. (Tianjin, China). Tigecycline (98.0 % purity, lot number A1013AS) and tetracycline standards (98.0 % purity, lot number N1014AS) were obtained from Dalian Meilun Biotechnology Co. Ltd. (Dalian, China) (Fig. 1). Acetonitrile (HPLC grade) was provided by Merck Company, Inc. (Darmstadt, Germany). Formic acid (HPLC grade) was purchased from Beijing MREDA Technology Co., Ltd. (Beijing, China). Water was obtained from an ultrapure water system manufactured by Millipore (Billerica, MA, USA). Plasma samples devoid of any antibiotics were obtained from individuals who had not previously taken OMA or TGC.

Instrumentation

The study utilized a UPLC-MS/MS setup (Thermo Scientific, San Jose, CA, USA), comprising an UltiMate 3000 UHPLC Liquid Chromatograph and Electrospray ionization source (ESI) on a Quantum Access® triple quadrupole mass spectrometer. The samples were prepared using a TG18-WS high-speed centrifuge (Drawell, China), a micropipette (Eppendorf, Hamburg, Germany), and Axygen ® polypropylene centrifuge tubes (Corning Ltd., Corning, NY, USA). Xcalibur ®2.2 SP1 software (Thermo Scientific, San Jose, CA, USA) was used for data acquisition, Thermo TSQ Tune Master ®2.3.0.1214 SP3 software (Thermo Scientific, San Jose, CA, USA) was used for optimization of mass spectrometry conditions, and LCquan ®2.8.0.51 software (Thermo Scientific, San Jose, CA, USA) was used for peak integral and calibration.

LC-MS/MS conditions

A Hypersil GOLD^{TM} C18 column (2.1 × 100 mm, 1.9 μm, Thermo Scientific, San Jose, CA, USA) was used for chromatographic separation. Flowing at 0.35 mL/min, solvent A consisted of 0.1 % formic acid in aqueous solution, whereas solvent B consisted of acetonitrile. The elution gradient steps included 0–0.5 min with 5 % B, 0.5–1.0 min with 5 %–18 % B, 1.0–1.8 min with 18 %–90 % B, 1.8–2.1 min with 90 % B, and 2.1–3.5 min with 5 % B. The chromatographic column and autosampler were held at 30 °C and 4 °C, respectively, and injections were 5 μL.

Positive ESI mode was used for mass spectrometry. An ion source temperature of 320 °C was set with a spray voltage of 3000 V. Nitrogen was employed as the shielding and auxiliary gas at pressures of 35 and 10 Arb, respectively. Approximately 1.5 mTorr of pressure was applied to the collision gas, which was argon. Selective reaction monitoring (SRM) for specific transitions was used to detect the OMA, TGC, and internal standards (IS) (Table 1). The data for this study were sourced from quantitative ions pairs.

Preparation of standard and quality control samples

A 0.2 % formic acid aqueous solution was used to dissolve and dilute OMA, TGC, and IS stock solutions based on their solubility and stability. Stock and prepared solutions were stored in a −80 °C freezer until needed. In the mixed standard stock solution, the concentrations of OMA and TGC were both 25,000 ng/mL. To obtain calibration curves and quality control (QC) samples, different batches of standard working

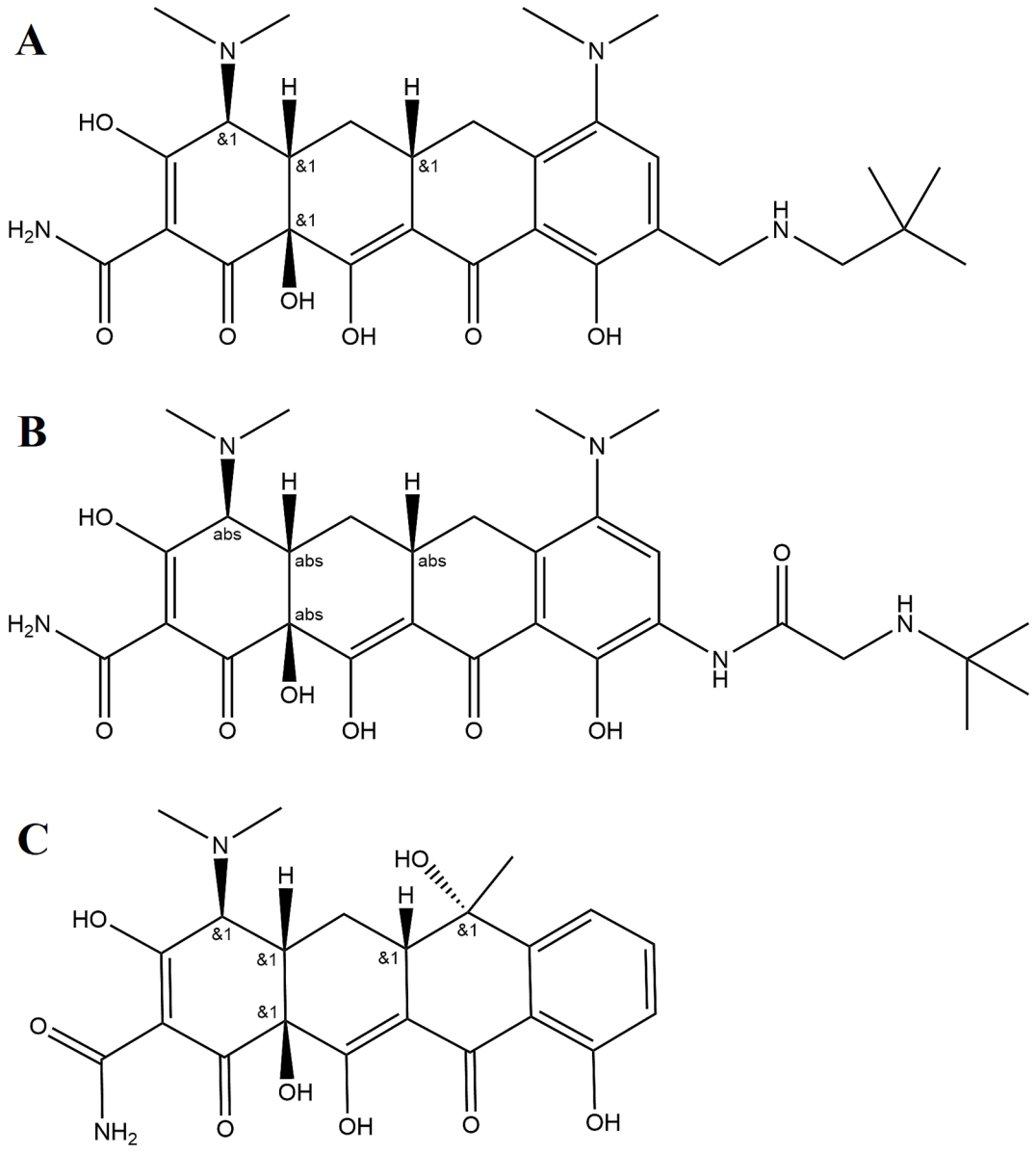

Fig. 1. Molecular structures of (A) omadacycline, (B) tigecycline, and (C) tetracycline.

Table 1
Method for quantification of plasma concentrations by LC-MS/MS.

Analytes	Mean RT [min]	Transitions [*m/z*] Parent → product	CE [V]	Tube Lens [V]
Omadacycline (quantitative analysis)	2.51	279.1 → 227.1	18	38
Omadacycline (qualitative analysis)	2.51	279.1 → 270.6	13	38
Tigecycline (quantitative analysis)	2.33	293.6 → 257.1	12	39
Tigecycline (qualitative analysis)	2.33	293.6 → 228.8	16	39
Tetracycline (quantitative analysis)	2.62	445.2 → 410.3	17	54
Tetracycline (qualitative analysis)	2.62	445.2 → 153.9	23	54

solutions were mixed with blank plasma. The standard working solution and blank plasma were mixed at a ratio of 1:9, and the concentration of the IS working solution was 2,000 ng/mL. The final concentrations of plasma samples containing analytes are listed in Table 2.

Sample preparation

The plasma samples of OMA and TGC (100 μL) were transferred to a centrifuge tube. Then, 10 μL of the IS working solution and 200 μL of acetonitrile were added, and the protein was precipitated by vortex mixing for 60 s. The mixture was then centrifuged at 14,500 rpm for five minutes. Subsequently, 100 μL of the supernatant was transferred to a 1.5 mL centrifuge tube. Meanwhile, 200 μL of a 0.2 % formic acid aqueous solution was added. The mixture was vortexed for 60 s and then centrifuged at 14,500 rpm for five minutes. For analysis, 5 μL of the supernatant was injected into the UPLC-MS/MS.

Method validation

This method was validated following the guidelines for the

Table 2
Calibration and quality control (QC) concentrations (ng/mL).

Analytes	Calibration concentration							QC concentrations			
	1	2	3	4	5	6	7	LLOQ	Low	Medium	High
Omadacycline	25	50	100	250	500	1000	2,500	25	75	400	2,000
Tigecycline	25	50	100	250	500	1000	2,500	25	75	400	2,000

verification of biological analysis methods provided by the FDA [21] and the 2020 edition of the Chinese Pharmacopoeia [22]. Validation of the method involved assessing selectivity, specificity, carryover, linearity, lower limit of quantitation (LLOQ), calibration curve, accuracy, precision, matrix effects, extraction recovery, stability, and solvent effects.

Selectivity, specificity, and carryover

Six different individual plasma samples (without OMA and TGC) were chosen to assess the specificity and selectivity of this assay, examining how endogenous substances and impurities in the plasma matrix impact the measurement of analytes. Typically, if the response of plasma samples (without OMA and TGC) is below 20 % of the analyte's LLOQ and 5 % of the IS, there will be no interference. Carryover was evaluated by sequential injection of LLOQ, upper limit of quantitation (ULOQ), and blank samples. The requirements for carryover were consistent with those for selective experiments.

Linearity and LLOQ

Linearity was evaluated by analyzing the standard curves at seven concentrations (25, 50, 100, 250, 500, 1000, and 2,500 ng/mL). To construct the calibration curve, the peak area ratio (analyte/internal standard) was used as the horizontal coordinate, while the ratio of plasma concentration served as the vertical coordinate. The calibration curve equation was determined through linear regression using the least squares method with $1/X^2$ as the weighting factor. The coefficient of determination (r^2) of all the standard curves should not be less than 0.9900. The LLOQ refers to the minimum amount of analyte that can be accurately and reliably measured in a sample. In addition to the LLOQ, the other standard concentrations should fall within ± 15 % of the nominal value, and the signal-to-noise ratio (S/N) needs to be a minimum of 10 for accurate and precise measurement. The deviation of the LLOQ was within ± 20 % based on the analysis of six replicates.

Precision and accuracy

By analyzing six replicates in a single run at three different quality control levels (low QC, medium QC, and high QC) and LLOQ, intra-day accuracy and precision were assessed. Three separate dates were utilized to analyze identical QC samples to evaluate the precision and accuracy between days. Accuracy was determined by comparing nominal and experimental concentrations, while precision was assessed by calculating the percentage coefficient of variation (CV%). The QC samples should demonstrate an average accuracy within ± 15 %, and the accuracy of the LLOQ should fall within a range of ± 20 %. Unless the LLOQ exceeded 20 %, each concentration level was accurate to within 15 %.

Matrix interference and recovery during extraction

The matrix impact was assessed by testing low, medium, and high-QC levels in six separate plasma samples with no analytes present. Matrix factor (MF) values for the analyte and IS were determined by dividing the peak area of the supernatant with the matrix by the peak area of the pure solution without the matrix. Analytes were evaluated using their MF ratios against the isoforms to determine the IS-normalized matrix factor (IS-MF). In the six matrix batches, CV% for the IS-MFs should not exceed 15 %. Analyzing the peak areas of samples spiked before and after extraction allowed for the calculation of the recovery rates of the analyte and IS.

Stability

Analyte stability was evaluated in plasma using three levels of QC samples stored under four different storage conditions. The freeze–thaw stability was assessed by subjecting the samples to three full freeze–thaw cycles (transitioning from −80 °C to 25 °C) at room temperature. Analyses were performed on QC samples that were stored at 25 °C for 24 h for a short-term stability assessment. The long-term stability of the plasma samples was analyzed after simultaneous preservation at −20 °C and −80 °C for 14 and 45 days. QC samples were extracted at 4 °C and stored in an automatic sampler for 24 and 48 h to assess post-process stability. Stability was determined by comparing the average analyte concentrations to the initial nominal concentrations, with stability defined as a percentage difference within ± 15.0 %.

Dilution effects

Diluted QC samples were created to confirm the dilution effect, which was achieved by mixing blank plasma with the initial solution. The concentrations of the diluted QC samples are listed in Table 2. The high-concentration plasma samples were extracted following a 10-fold dilution with blank plasma and tested.

Applicability of the method for routine TDM

Our antimicrobial management plan was followed when applying this technique to clinical samples obtained from severely ill patients receiving OMA or TGC. All patients had critically ill infections caused by multidrug-resistant (MDR) *A. baumannii*. OMA administration and dosage involved starting with an initial intravenous dose of 200 mg, followed by a daily dose of 100 mg. TGC was administered through an intravenous drip beginning with 100 mg, followed by 50 mg every 12 h. The antimicrobial infusion time was set at 60 min, and the duration of therapy was at least five days. Patient samples were collected after at least the fifth dose, with the assumption that steady-state plasma concentrations had been reached. If the patient's initial dose was doubled, blood samples were collected after the fourth dose. Peripheral blood containing OMA and TGC was collected 15 min before administration in K_2-EDTA vacuum-collection tubes. After centrifuging at 5000 rpm for five minutes at room temperature, the plasma was separated and promptly stored at −80 °C.

Results and discussion

Method development

Optimizing the ionization and fragmentation conditions of the analytes and IS led to the development of a chromatographic method that is both symmetric and sharp-peaked, with excellent selectivity and sensitivity. Tetracycline was selected as the IS, and scanning was performed using an ESI ion source in positive ion mode selective reaction monitoring (SRM). The quantitative ion pairs for OMA and TGC were *m/z* 279.1 → 227.1 and 293.6 → 257.1, respectively (Table 1). The production mass spectrum for the IS was obtained using the precursor ion *m/z* 445.2 [M + H]+, with the most abundant fragment found at *m/z* 410.3.

Several combinations of mobile phases and different additive concentrations were evaluated to achieve adequate separation, peak symmetry, and response. Acetonitrile was tested as the organic mobile

phase, and formic acid was used to improve the ionic strength. Due to the small particle size (1.9 μm), a flow rate of 0.35 mL/min was selected to ensure that the column pressure remained within the normal range. A gradient elution program was established, and the total running time was less than 3.5 min. This research introduced a novel UPLC-MS/MS technique to quantify OMA and fine-tuned the method for TGC analysis. The method requires only a 50 μL plasma sample, which is very convenient, practical, and rapid.

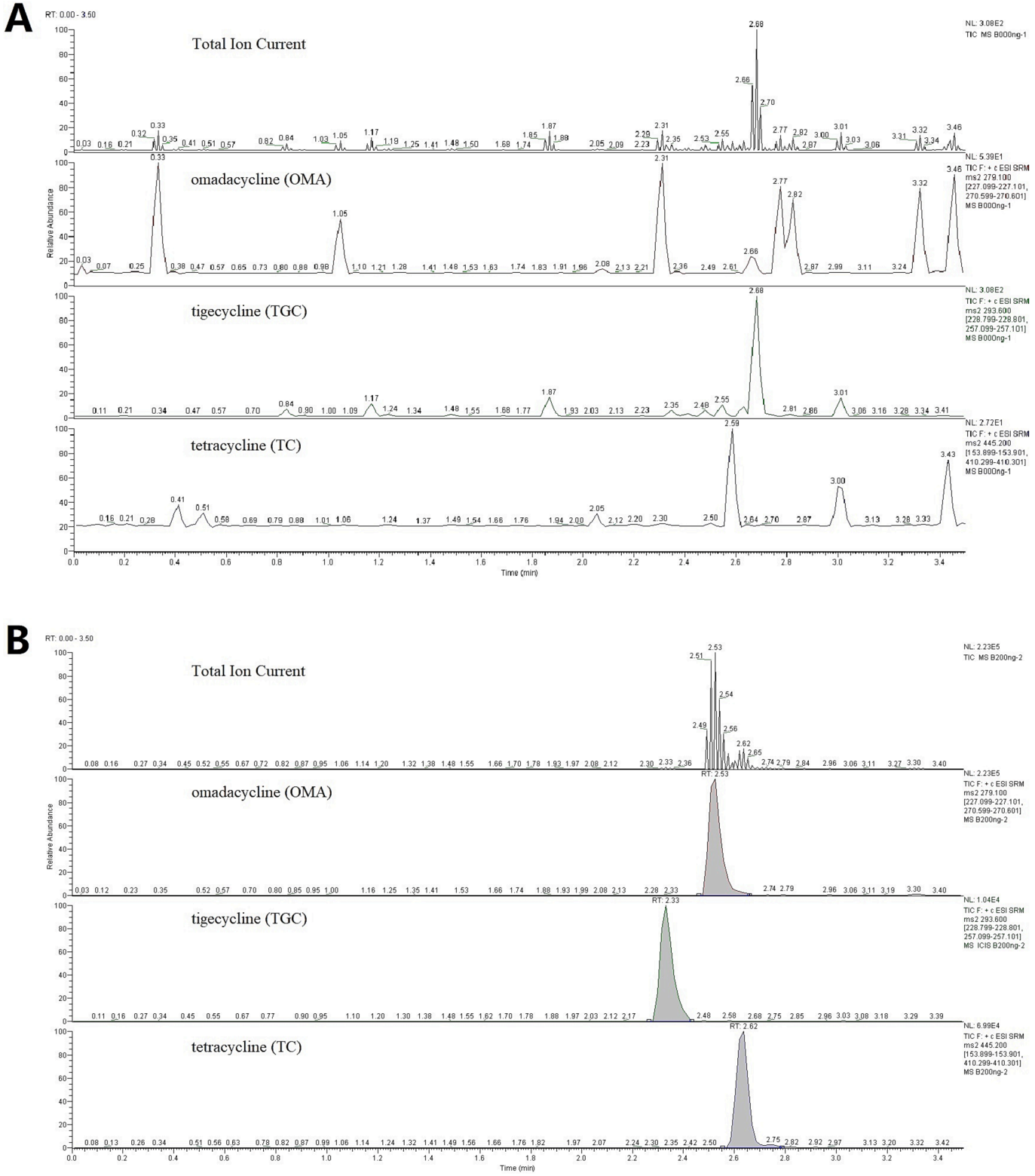

Fig. 2. Examples of extracted ion chromatograms of drug-free plasma spiked with: (A) nothing, showing blank SRM channels for Total Ion Current (TIC), omadacycline (OMA), tigecycline (TGC), and tetracycline (TC), (B) TIC, OMA, TGC, TC. Note: Concentrations of OMA, TGC, and TC were all 200 ng/ml.

Method validation

Selectivity, specificity, and carry-over

Comparisons were made between the extracted ion chromatograms of identical substrates to confirm the absence of interference from endogenous substances or other components. The overall run duration was 3.5 min, with OMA, TGC, and IS exhibiting average retention times of 2.53, 2.33, and 2.62 min, respectively. Fig. 2 displays chromatograms of human plasma without any substances, plasma samples with tetracycline calibration (200 ng/mL), and plasma samples with OMA and TGC (200 ng/mL). During the retention time for analyte and IS, no endogenous interference was detected, leading to the conclusion that the selectivity was acceptable. In the blank plasma run after the ULOQ, negligible peaks of TGC and tetracycline were observed, indicating the absence of TGC and tetracycline residues. However, a residual effect of OMA was noted; after injecting a blank sample, the residue of OMA was lower than the 20 % LLOQ and 5 % IS.

Linearity and LLOQ

A strong linear relationship was observed for OMA and TGC in human plasma across concentrations ranging from 25 to 2,500 ng/mL. The calibration curves for both OMA and TGC achieved linear regression fitting, demonstrating a strong correlation between the measured concentrations and the corresponding peak area ratios. ($r^2 > 0.995$; Table 3). The range of 25–2,500 ng/mL was sufficiently linear to determine pharmacokinetic parameters of OMA and TGC in critically ill patients receiving standard or high-dose treatments, as most concentrations fell within this range. The ULOQ may be used in the treatment of high-dose TGC, as concentrations above 3,000 ng/mL may be observed during the elimination phase of TGC [23]. According to the literature, when the concentration is lower than the LLOQ, a larger sample volume is required, or plasma samples need to be treated with concentration following protein pretreatment [24]. No concentrations below the LLOQ were found in the current study, hence, there was no need for a minimum quantification threshold for TDM of TGC. None of the standard concentrations differed from the theoretical values by ±15 %. The signal-to-noise (S/N) ratio for each LLOQ was >10.

Precision and accuracy

To assess the exactness and correctness of the technique, QC samples at three different concentration levels were analyzed six times on either the same day (intra-day) or three separate days (inter-day). The intra-day and inter-day accuracies are listed in Table 4. The intra-day precision of the four OMA concentration levels ranged from 2.81 % to 8.05 %, and the accuracy range was between 103.31 % and 106.85 %. The inter-day precision varied from 2.86 % to 7.34 %, with accuracy between 99.94 % and 106.32 %. The precision of the TGC LLOQ and QC samples at low, medium, and high levels varied between 95.71 % and 107.82 %. The precision for intra- and inter-day analyses ranged from 3.05 % to −8.77 % and from 6.32 % to −7.67 %, respectively. These findings indicate that this approach is dependable and consistent and can be reliably used for the quantitative analysis of OMA and TGC in human plasma.

Table 3
Linear ranges, linear equations, correlation coefficients, and LLOQs of OMA and TGC.

Analytes	Internal standard	Linear range (ng/mL)	Regression equation	R^2	LLOQ (ng/mL)
Omadacycline (OMA)	Tetracycline	25–2,500	Y = -0.052588 + 22.1461*X	0.9992	25
Tigecycline (TGC)		25–2,500	Y = -0.0134651 + 0.974946*X	0.9984	25

Matrix effects and extraction recovery

A summary of the matrix effects and extraction recovery results can be found in Table 5. The IS-normalized MFs for OMA were between 86.22 % and 91.12 % and the CV was less than 7.92 %. The IS-normalized MFs for TGC was 87.72 %–90.80 %, and the CV was lower than 15 %. The extraction recoveries of OMA and TGC were 92.65 %–99.73 % and 92.88 %–101.70 % respectively, under low QC, medium QC, and high QC levels. Based on these results, it is expected that protein precipitation can be used to pre-treat plasma samples to increase extraction efficiency without compromising matrix integrity.

Stability

The results of short-term stability, long-term frozen stability, freeze–thaw stability, and automatic injector stability of the OMA and TGC plasma samples are shown in Table 6, with accuracy not exceeding ± 12.0 %. The stability of OMA and TGC in the extracted plasma samples was maintained for 24 h at room temperature and for 48 h in an automatic injector at 4 °C. Both OMA and TGC displayed acceptable stability at −20 °C and −80 °C for 45 days. In this study, the authors found that OMA plasma samples remained stable after being frozen and thawed three times between −80 °C and room temperature, with TGC showing the same results, consistent with previous reports [25]. This indicates that OMA and TGC are stable under these storage conditions.

Dilution effects

The QC samples were diluted 10 times with a blank plasma sample to evaluate dilution integrity. The precision of OMA and TGC was found to be between 2.5 % and 5.56 %, while the accuracy ranged from 92.71 % to 108.1 %. The results demonstrated that the integrity of a 10-fold dilution was reliable and that samples exceeding the calibration curve's range could be accurately determined following dilution.

Solvent effect

Neither OMA nor TGC exhibited solvent effects during the establishment of the analytical method. However, the solvent effect of TGC was significant (Fig. 3). To remove the solvent impact of TGC, dilute the supernatant post-protein precipitation by more than 1.5 times with water containing 0.2 % formic acid. The solvent effect during the methodological validation process may be due to the high proportion of acetonitrile in the supernatant after protein precipitation. The initial mobile phase A ratio used is 5 %. After protein precipitation, the supernatant was aspirated and diluted 1.5x the volume with a 0.2 % formic acid aqueous solution, successfully eliminating the solvent effect of TGC.

Routine TDM application of the method

The pathophysiological functions of critically ill patients are complex and often complicated by capillary leakage, tissue edema, hypoproteinemia, multiple organ failure, and the use of external organ function support therapies such as continuous renal replacement therapy (CRRT) and extracorporeal membrane oxygenation (ECMO). The altered physiological conditions in critically ill patients can impact the disposition of antibiotics [26,27], complicating the ability to forecast drug levels in the blood and posing challenges for the appropriate use of antibiotics. Hence, it is crucial to assess their exposure in critically ill individuals to ascertain successful treatment outcomes [28]. Blood samples for OMA (n = 6) and TGC (n = 3) were analyzed using this UPLC-MS/MS technique. For one of the patients with MDR *Acinetobacter baumannii* infection, sputum culture results showed sensitivity to TGC [minimum inhibitory concentration (MIC) = 2 μg/mL]. The treatment team first administered the prescribed dose of OMA for eight days, but infection control was poor. The dose was then changed to twice the prescribed dose of TGC. After seven days of treatment, the infection was controlled. This special case suggests that the dosage based on drug

Table 4
Stability results of OMA and TGC in plasma at different storage conditions (%, n = 6).

Analytes	Nominal Concentrations (ng/mL)	Intra-day (%, n = 6)		Inter-day (%, n = 18)	
		Precision	Accuracy	Precision	Accuracy
Omadacycline (OMA)	25	8.05	106.85	7.34	104.22
	75	3.22	103.31	4.13	99.94
	400	2.81	104.29	2.86	100.16
	2,000	4.10	106.64	3.68	106.32
Tigecycline (TGC)	25	3.05	96.53	6.94	104.57
	75	8.77	98.76	7.67	95.71
	400	6.50	106.15	7.35	98.41
	2,000	3.83	107.82	6.32	106.43

Table 5
Matrix effects and extraction recoveries of OMA and TGC in human plasma (mean ± RSD, n = 6).

Analytes	Nominal Concentrations (ng/mL)	Extraction recovery (%)	Matrix effect (%)
Omadacycline (OMA)	75	97.64 ± 8.89	91.12 ± 7.92
	400	92.65 ± 8.72	86.22 ± 2.54
	2,000	99.73 ± 6.71	87.55 ± 2.00
Tigecycline (TGC)	75	94.71 ± 5.74	87.72 ± 3.07
	400	92.88 ± 7.74	90.80 ± 5.55
	2,000	101.70 ± 7.01	88.39 ± 2.85

instructions may not be accurate, and TDM for administering antibiotics to patients with severe infections has important clinical significance.

Table 7 summarizes the specific time required to obtain the sample, antibiotic dosage, and trough concentrations. The results showed that the concentrations of OMA and TGC differed among patients administered the same dose. Although the measured drug levels correlated with the MIC of the antibiotic against the resulting bacterial pathogen assessed *in vitro*, each patient's treatment was optimized according to a specific pharmacokinetic/pharmacodynamic (PK/PD) index [29]. This method provides a theoretical basis for optimizing the administration of OMA and TGC to critically ill patients to improve their anti-infection effect and clinical prognosis. Currently, no methods for monitoring blood concentration levels of OMA have been reported. This research is pioneering the development of a UPLC-MS/MS technique to measure OMA levels in human plasma and can also be used for analyzing PK in critically ill individuals. This tool will effectively provide evidence for personalized antibiotic treatment guided by real-time TDM in critically ill patients.

Conclusion

Currently, there are no reports on the monitoring of OMA concentration. Nevertheless, this research has developed a quick, sensitive, and uncomplicated technique for extracting and measuring OMA and TGC in human plasma. The method demonstrated acceptable specificity, precision, accuracy, and linearity in the range of 25–2,500 ng/mL. Our method offers significant advantages, including a small injection volume (5 μL) and a short run time (3.5 min). The UPLC-MS/MS system is suitable for analyzing clinical plasma samples. This platform will aid in assessing the effectiveness and safety of OMA and TGC exposure in critically ill patients for upcoming PK/PD research.

Funding statement

This study was supported by the Cuiying Scientific and Technological Innovation Program of The Second Hospital & Clinical Medical School, Lanzhou University (CY2023-QN-A04), Gansu Provincial Department of Science and Technology, Soft Science Special Project (23JRZA485), and Lanzhou Science and Technology Development Guiding Plan Project

Table 6
Results of the sample storage stability experiment (n = 6).

Analytes	Times	Nominal Concentrations (ng/mL)	RT (%)	+4 °C (%)	−20 ℃ (%)	−80 ℃ (%)	RFT (%)
Omadacycline (OMA)	24 h	75	−5.67	−2.33	–	–	–
		400	−3.12	−3.29	–	–	–
		2,000	−4.46	6.04	–	–	–
	48 h	75	–	8.18	–	–	–
		400	–	3.33	–	–	–
		2,000	–	7.09	–	–	–
	14d	75	–	–	3.72	5.38	7.17
		400	–	–	−1.20	3.44	6.06
		2,000	–	–	7.36	8.19	4.29
	45d	75	–	–	−8.70	−7.59	–
		400	–	–	−3.14	−6.02	–
		2,000	–	–	3.56	2.94	–
Tigecycline (TGC)	24 h	75	−4.58	−4.21	–	–	–
		400	−5.47	−3.24	–	–	–
		2,000	1.61	−2.80	–	–	–
	48 h	75	–	6.69	–	–	–
		400	–	4.26	–	–	–
		2,000	–	5.58	–	–	–
	14d	75	–	–	4.38	7.53	6.05
		400	–	–	8.38	4.53	5.49
		2,000	–	–	8.80	6.88	7.17
	45d	75	–	–	8.79	−0.22	–
		400	–	–	5.82	4.35	–
		2,000	–	–	9.29	11.73	–

Note: room temperature (RT); repeatedly frozen and thawed (RFT). At 75, 400, and 2,000 ng/mL, the mean % difference from the original nominal concentrations (measured at T0) of the 3 QC levels (low (L), medium (M) and high (H)) is represented by each time point.

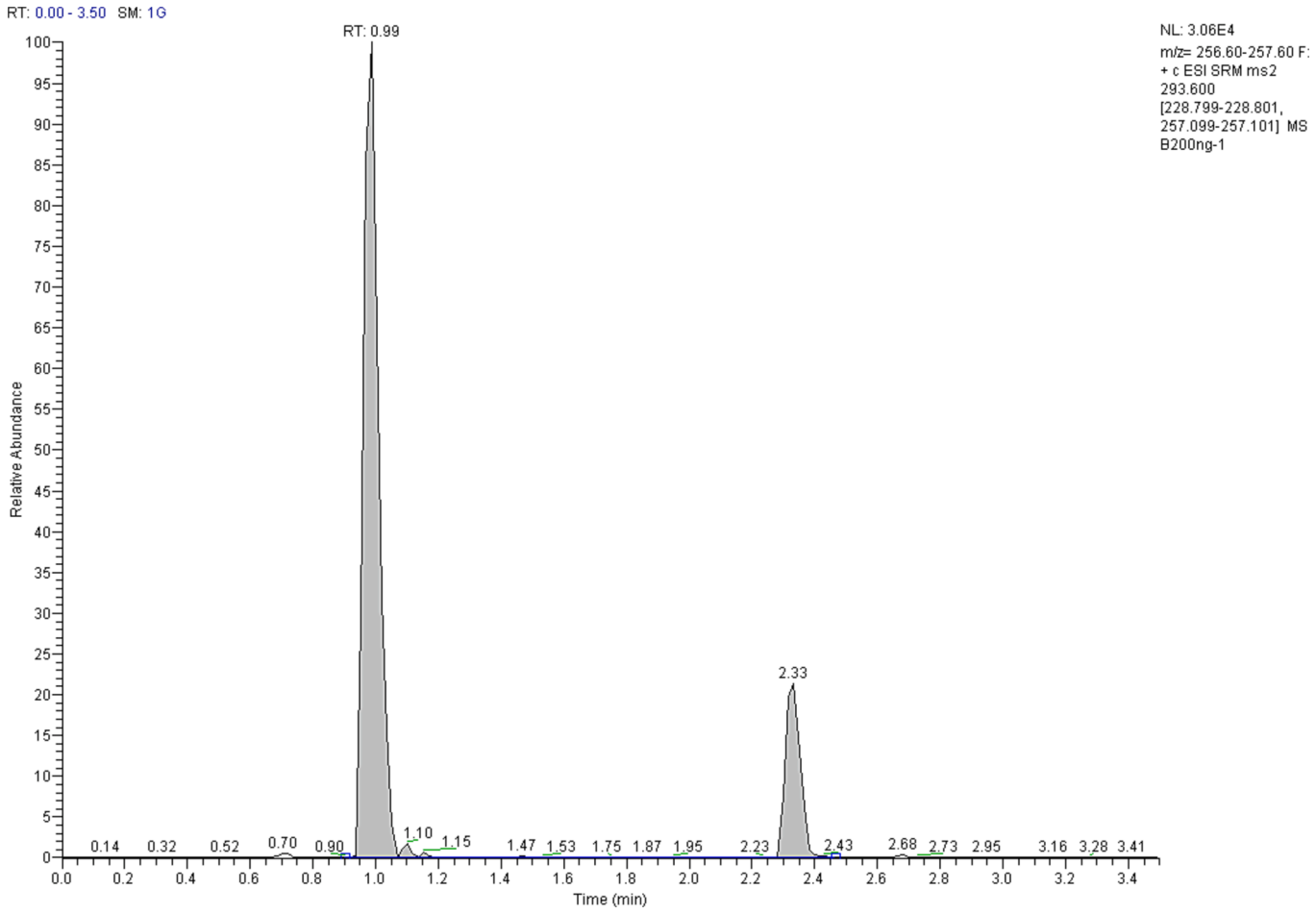

Fig. 3. Tigecycline (TGC) solvent effect: chromatographic profile of a 200 ng/mL plasma sample.

Table 7
Results of therapeutic drugs monitoring of OMA and TGC in severely patients with community acquired pneumonia infection.

NO.	Sex	Age	Days of administration	Drug administration plan	Trough concentration of plasma(ng/mL)	
					Omadacycline	Tigecycline
1	male	71	7	loading with 200 mg, maintaining at 100 mg qd	180	–
2	female	25	5		160	–
3	male	51	6	100 mg, qd	190	–
4	male	41	4		120	–
5	male	75	5		110	–
6	male	73	8		130	
7	male	68	4	loading with 100 mg, maintaining at 50 mg q12h	–	584
8	male	88	7		–	228
9	male	73	7	loading with 200 mg, maintaining at 100 mg q12h	–	799

(2022-5-131).

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the Second Hospital & Clinical Medical School, Lanzhou University (Approval number 2024A-034). Before participating in the study, all patients provided informed consent for inclusion.

CRediT authorship contribution statement

Chang Wang: Writing – review & editing, Writing – original draft, Funding acquisition, Data curation, Conceptualization. **Bingfeng Luo:** Writing – original draft, Visualization, Data curation. **Wenqing Liu:** Writing – original draft, Investigation. **Chen Jia:** Project administration. **Haile Chen:** Investigation. **Jingjing Ma:** Investigation. **Xia Song:** Writing – original draft. **Xingfang Ji:** Methodology, Investigation. **Aijia Cao:** Methodology, Investigation. **Yinliang Bai:** Project administration, Conceptualization. **Wen Qiu:** Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank all participants who were enthusiastically involved in this research. They are also thankful for their great support from The Second Hospital & Clinical Medical School, Lanzhou University Therapeutic Drug Monitoring Group of the Department of Pharmacy.

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

References

[1] Ten threats to global health in 2019. Geneva: World Health Organization; 2019 (https://www.who.int/news-room/spotlight/ten-threats-to-globalhealth-in-2019, accessed 10 Janusry 2024).
[2] C.J. Murray, K.S. Ikuta, F. Sharara, et al., Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis[J], Lancet 399 (10325) (2022) 629–655, https://doi.org/10.1016/s0140-6736(21)02724-0.
[3] T. Thompson, The staggering death toll of drugresistant bacteria[J], Nature (2022), https://doi.org/10.1038/d41586-022-00228-x.
[4] Institute for Health Metrics and Evaluation. Drugsusceptible tuberculosis—Level 4 cause. In: Global burden of disease summaries. Institute for Health Metrics and Evaluation; 2020 (https://www.healthdata.org/results/gbd_summaries/2019/drugsusceptible-tuberculosis-level-4-cause, accessed 10 Janusry 2024).
[5] Drug-resistant infections: a threat to our economic future. 2017 , 2. (https://documents1.worldbank.org/curated/en/323311493396993758/pdf/final-report.pdf, accessed 10 Janusry 2024).
[6] J. O'Neill, Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. Review on Antimicrobial Resistance. Wellcome Trust and HM Government. 2016. (https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf, accessed 10 Janusry 2024).
[7] Drug-resistant infections: a threat to our economic future. Washington, D.C.: World Bank; 2017. License: Creative Commons Attribution CC BY 3.0 IGO (http://documents.worldbank.org/curated/en/323311493396993758/final-report, accessed 10 Janusry 2024).
[8] P. Czuppon, T. Day, F. Débarre, A stochastic analysis of the interplay between antibiotic dose, mode of action, and bacterial competition in the evolution of antibiotic resistance[J], PLoS Comput. Biol. 19 (8) (2023) e1011364, https://doi.org/10.1371/journal.pcbi.1011364.
[9] J.M. Dulhunty, D. Paterson, S.A. Webb, et al., Antimicrobial utilisation in 37 Australian and New Zealand intensive care units[J], Anaesth. Intensive Care 39 (2) (2011) 231–237, https://doi.org/10.1177/0310057X1103900212.
[10] M. Campion, G. Scully, Antibiotic use in the intensive care unit: optimization and de-escalation[J], J. Intensive Care Med. 33 (12) (2018) 647–655, https://doi.org/10.1177/0885066618762747.
[11] J.A. Roberts, S.K. Paul, M. Akova, et al., DALI: defining antibiotic levels in intensive care unit patients: are current β-lactam antibiotic doses sufficient for critically ill patients? [J], Clin. Infect. Dis. 58 (8) (2014) 1072–1834, https://doi.org/10.1093/cid/ciu027.
[12] C.D. Sumi, A.J. Heffernan, J. Lipman, et al., What antibiotic exposures are required to suppress the emergence of resistance for gram-negative bacteria? A systematic review[J], Clin. Pharmacokinet. 58 (11) (2019) 1407–1443, https://doi.org/10.1007/s40262-019-00791-z.
[13] M.E. Ibrahim, Prevalence of acinetobacter baumannii in Saudi Arabia: risk, factors, antimicrobial resistance patterns and mechanisms of carbapenem resistance[J]. Ann. Clin. Microb. Anti 18(1):1. DOI: 10.1186/s12941-018-0301-x.
[14] L.K. Logan, R.A. Weinstein, The epidemiology of carbapenem-resistant enterobacteriaceae: the impact and evolution of a global menace[J], J Infect Dis 215 (suppl_1) (2017) S28–S36, https://doi.org/10.1093/infdis/jiw282.
[15] S. Yaghoubi, A.O. Zekiy, M. Krutova, et al., Tigecycline antibacterial activity, clinical effectiveness, and mechanisms and epidemiology of resistance: narrative review[J], Eur. J. Clin. Microbiol. Infect Dis. 41(7) (2202) 1003–1022.
[16] M. Yu, S. Wu, B. Qi, et al., Rational use of tigecycline and tigecycline blood concentration monitoring in patients with severe infection[J], Biomed Rep. 19 (2) (2023) 51, https://doi.org/10.3892/br.2023.1634.
[17] X.R. Bai, Z.Z. Wang, W.C. Li, et al., Clinical efficacy and safety of tigecycline based on therapeutic drug monitoring for carbapenem-resistant Gram-negative bacterium pneumonia in intensive care units[J], BMC Infect. Dis. 23 (1) (2023) 830, https://doi.org/10.1186/s12879-023-08815-7.
[18] G.G. Zhanel, J. Esquivel, S. Zelenitsky, et al., Omadacycline: a novel oral and intravenous aminomethylcycline antibiotic agent[J], Drugs 80 (3) (2020) 285–313, https://doi.org/10.1007/s40265-020-01257-4.
[19] Peter S.A. Glass, MB, ChB, FFA(SA), Tong J. Gan, MB, BS, FRCA, FFARCSI, Scott M. D. Howell, A review of the pharmacokinetics and pharmacodynamics of remifentanil, Anesthesia Analgesia 89(4S): p 7, October 1999. | DOI: 10.1097/00000539-199910001-00003.
[20] G. Sakoulas, M. Nowak, M. Geriak, Omadacycline in treating community-based infections: a review and expert perspective, Expert Rev. Anti Infect. Ther. 21 (3) (2023 Mar) 255–265, https://doi.org/10.1080/14787210.2023.2174100.
[21] FDA, Bioanalytical method validation guidance for industry, 2018. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/bioanalytical-method-validation-guidance-industry.
[22] Chinese Pharmacopoeia Commission, "Guidelines for validation of methods for quantitative analysis of biological samples," in Pharmacopoeia of the people's republic of China. Beijing, China Medical Science Press, 2020, 466-472.
[23] G. De Pascale, L. Lisi, G.M.P. Ciotti, et al., Pharmacokinetics of high-dose tigecycline in critically ill patients with severe infections[J], Ann. Intensive Care 10 (1) (2020) 94, https://doi.org/10.1186/s13613-020-00715-2.
[24] J. Xie, T. Wang, X. Wang, et al., Quantitative analysis and pharmacokinetics study of tigecycline in human serum using a validated sensitive liquid chromato-graphy with tandem mass spectrometry method[J], J. Sep. Sci. 37 (12) (2014) 1396–1403, https://doi.org/10.1002/jssc.201400152.
[25] S. Mei, X. Luo, X. Li, et al., Development and validation of an LC-MS/MS method for the determination of tigecycline in human plasma and cerebrospinal fluid and its application to a pharmacokinetic study[J], Biomed. Chromatogr. 30 (12) (2016) 1992–2002, https://doi.org/10.1002/bmc.3776.
[26] M.H. Abdul-Aziz, J.-W. Alffenaar, M. Bassetti, et al., Antimicrobial therapeutic drug monitoring in critically ill adult patients: a position paper[J], Intensive Care Med. 46 (6) (2020) 1127–1153, https://doi.org/10.1007/s00134-020-06050-1.
[27] K.L. Maguigan, M.H. Al-Shaer, C.A. Peloquin, Beta-lactams dosing in critically ill patients with gram-negative bacterial infections: a PK/PD approach[J], Antibiotics (Basel) 10 (10) (2021) 1154, https://doi.org/10.3390/antibiotics10101154.
[28] L.A. Decosterd, T. Mercier, B. Ternon, et al., Validation and clinical application of a multiplex high performance liquid chromatography - Tandem mass spectrometry assay for the monitoring of plasma concentrations of 12 antibiotics in patients with severe bacterial infections (vol 1157, 122160, 2020)[J], J. Chromatogr., B. Anal. Technol. Biomed. Life Sci. 2022.
[29] L. Evans, A. Rhodes, W. Alhazzani, et al., Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021, Intensive Care Med. 49 (11) (2021) e1063–e1143, https://doi.org/10.1097/CCM.0000000000005337.