INTRODUCTION

Cefepime is a 4th-generation cephalosporin with activity against a range of facultative Gram-positive and Gram-negative bacteria [1]. Due to its broad spectrum of activity, it is frequently used as empiric antibiotic coverage in patients suspected to have serious bacterial infections, such as critically ill and immunocompromised patients. It is also administered as definitive treatment for infections caused by Gram-negative bacilli resistant to narrower spectrum antibiotic agents, including Enterobacteriaceae that produce AmpC β-lactamases [2] and many strains of Pseudomonas aeruginosa [3]. As a result, cefepime has become an important antimicrobial agent in the treatment of serious community- and hospital-acquired bacterial infections. The pharmacokinetics (PK) of cefepime have been well-characterized. It is renally eliminated with greater than 80% of doses excreted unchanged in the
Cefepime demonstrates time-dependent bactericidal activity in which its efficacy is defined by the fraction of time for which the free (unbound) concentration is maintained above the minimum inhibitory concentration (MIC) of the bacteria being treated. The classic pharmacodynamic (PD) target that is associated with improved clinical outcomes for cefepime has been maintenance of drug concentrations above the MIC (\(FT_{MIC}\)) for \(\geq 60\%\) of dosing interval [10]. However, more recent data have suggested that more aggressive PD targets, maintaining drug concentrations above the MIC for the entire dosing interval (100\% \(FT_{MIC}\)), should be used to ensure optimal clinical and microbiological outcomes [11,12]. Successful treatment of harder to treat infections, such as Gram-negative pneumonia, may require the administration of doses that maintain free serum concentrations at least 2-fold higher than the MIC for the entire dosing interval (100\% \(FT_{2xMIC}\), or \(FC_{min}>2xMIC\)) [13]. Meanwhile, in vitro studies have demonstrated that suppression of resistance selection during therapy occurs with an even more robust PD target of \(FC_{min}>3.8xMIC\) [14]. These latter data are consistent with in vitro time-kill studies for \textit{Pseudomonas aeruginosa} that show increased bacterial killing for \(\beta\)-lactam agents at concentrations up to 4 times the MIC [15]; based on these time-kill data, some studies have used \(FT_{4xMIC}\) as the optimal PD target for \(\beta\)-lactam antibiotics [12].

In 2014, the Clinical and Laboratory Standards Institute (CLSI) revised cefepime interpretive criteria (breakpoints) for the treatment of Enterobacteriaceae based on data showing clinical failures and low probability of target attainment using standard cefepime dosing in adults infected with Gram-negative bacteria with an MIC of 4 and 8 µg/mL [16, 17]. To optimize the administration of cefepime, CLSI employed the designation of “susceptible-dose dependent” (SDD) for isolates with an MIC of 4 and 8 µg/mL [17]. This change was made to signal that dosing regimens that result in higher drug exposures (i.e. higher doses or more frequent dosing) be administered to give the highest probability of adequate coverage of these isolates [17]. Instead of using the term “intermediate”, which is often clinically interpreted as “resistant,” the SDD designation was implemented to encourage the administration of cefepime at high doses rather than discourage its use for treatment of less susceptible isolates. The SDD designation does not apply to \textit{Pseudomonas aeruginosa} and other non-Enterobacteriaceae Gram-negatives since the approved dosing regimen for treatment of these organisms differ [17].

There is accumulating evidence in critically ill adults that standard \(\beta\)-lactam dosing by intermittent infusion, including for cefepime, often produces inadequate antibiotic concentrations contributing to suboptimal outcomes [12,18-19]. Pediatric studies also have found that traditional dosing via intermittent infusion may be inadequate for less susceptible Gram-negative pathogens [20]. As a result, there is increased acceptance that administration of \(\beta\)-lactam agents as an extended or continuous infusion maximizes the likelihood of PD target attainment in critically ill patients [21]. The administration of cefepime as a prolonged infusion significantly increases the probability of PD target attainment compared to intermittent infusion, even for isolates in the SDD range [20,22]. A recent meta-analysis of 6 randomized controlled trials (RCTs) and 4 observational studies found that prolonged infusion of meropenem was associated with higher clinical success rate (odds ratio 2.10, 95% CI 1.31-3.38) and lower mortality (risk ratio 0.66, 95% CI 0.50-0.88).
in adult patients with severe infections compared to intermittent infusion [23]. Similarly, in a meta-analysis of 13 RCTs comparing continuous infusion versus intermittent bolus of β-lactam agents in critically adult patients with respiratory infections, continuous infusion was associated with higher cure rates (risk ratio 1.18, 95% CI 1.07-1.30) [24]. Although data specific to cefepime are limited, there is mounting evidence that the use of prolonged infusions improves the likelihood of PD target attainment, which is especially important in patients with critical illness, serious bacterial infections, and infections caused by isolates with decreased susceptibility to the β-lactam agent being administered.

The production of β-lactamases is the most frequent and important mechanism of resistance to β-lactam antibiotics among Gram-negative bacteria. Extended-spectrum β-lactamases (ESBLs), carbapenemases, and AmpC β-lactamases have the capacity to hydrolyze drugs in this class with SHV-, OXA- and CTX-M-type enzymes being most prominent among Pseudomonas aeruginosa and Enterobacteriaceae [25,26]. CTX-M-15 is the most widely distributed CTX-M-type ESBL and isolates producing this enzyme have higher MICs to cefepime than other ESBLs [27]. As noted above, maintenance of drug concentrations 4-fold above the MIC for the entire dosing interval decreases selection of resistance during cefepime therapy in a hollow-fiber infection model [14]. Although in vivo studies are needed to validate this target, optimized antibiotic exposures can prevent, or at least delay, the development of resistance among Gram-negative bacteria during treatment, particularly in high-burden infections such as pneumonia or undrained, intra-abdominal abscesses.

While administration of large doses can facilitate achievement of higher drug concentrations, cefepime is associated with dose-dependent toxicity particularly neurotoxicity [28-31], especially in patients with renal dysfunction, limiting the administration of overly large doses. In a single-center retrospective study of adult patient receiving cefepime who underwent therapeutic drug monitoring (TDM), a neurologic event considered possibly related to cefepime occurred in 11% of 93 patients [29]; patients with $C_{\text{min}}>20$ mg/L had a 5-fold higher risk for neurologic events (OR 5.1, 95% CI 1.3-19.8) and those with a $C_{\text{min}}>40$ mg/L had a 9-fold higher risk (OR 9.4, 95% CI 2.2-39.5). Decreased renal function was significantly associated with neurotoxicity [29]. Similarly, in a study of adult patients receiving cefepime for febrile neutropenia, high cefepime concentrations were an independent predictor of neurological toxicity with a 50% probability of toxicity at $C_{\text{min}}>22$mg/L [30]. Patients with acute or chronic renal dysfunction may have reduced clearance of cefepime, depending on the degree of glomerular filtration impairment, and are at increased risk of exposure-dependent neurotoxicity.

It is crucial to individualize cefepime dosing in order to achieve concentrations that optimize efficacy, limit selection of resistant bacteria, and avoid toxicity. This is especially true for critically ill patients and those with Gram-negative infections caused by less susceptible isolates who are at high risk of suboptimal drug exposures and resultant clinical failure, as well as patients with renal dysfunction at higher risk for cefepime-induced neurotoxicity. Monitoring of blood concentrations is particularly important in order to guide dosing that will achieve targeted serum drug concentrations. Although data demonstrating improved clinical outcomes with TDM for β-lactam agents are limited, TDM has been shown to improve PD target attainment in critically ill patients for various drugs in this class [32-34]. As a result, β-lactam TDM is becoming an increasingly valued part of clinical care of critically ill patients [21, 35-36].

In order to facilitate TDM, quantitative drug assays need to provide precise results with short turnaround time. For cefepime, drug concentrations are most often measured using validated high-performance liquid chromatography (HPLC) or liquid chromatography-tan-
dem mass spectrometry (LC-MS/MS) methods [37-40]. Cefepime poses an added challenge for TDM due to rapid ex-vivo degradation in plasma at room temperature, requiring that all processing occur at 4°C [39]. The purpose of this review is to list the various LC-MS/MS methods in human blood, plasma, and serum for quantitation of cefepime with information on sample preparation, chromatographic and mass spectrometric conditions and validation parameters (Table 1) and to discuss relevant bioanalytical strategies and considerations for implementation of LC-MS/MS assays for therapeutic drug monitoring (TDM) of cefepime.

**LC-MS/MS methods for cefepime analysis**

Cefepime (Figure 1, CAS no. 88040-23-7, Pyrrolidinium, 1-[[6R,7R)-7-[[2Z)-(2-aminoo-4-thiazolyl)(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl]-1-methyl-, inner salt) has a chemical formula of C_{19}H_{24}N_{6}O_{5}S_{2} with an average molecular weight of 480.56 g/mol.

**Sample preparation**

Sample preparation is a critical step in bioanalytical method development. Due to limited stability of cefepime in plasma and serum at room temperature, samples should be immediately placed on ice after collection and processed at 4˚C temperature to provide accurate results. Plasma [41,42] and serum [43-45] samples have been used as the biological matrix for cefepime TDM assays.

Sample cleanup by removal of interfering matrix components (proteins, salts, and lipids) is often necessary to reduce the risk of matrix effects in LC-MS/MS procedures and to provide required selectivity, sensitivity, and ruggedness. The two most common methods for sample cleanup are (i) protein precipitation and (ii) solid-phase extraction (SPE) for cefepime TDM assays. For protein precipitation, the majority of the reported assays utilize methanol or acetonitrile as the precipitation and extraction solvents. SPE with Oasis HLB cartridges was utilized by Ohmori et al. [43], and an online SPE cleanup with Oasis HLB column was employed by Zander et al. [45]. These assays utilized stable labeled 2D_{3}-cefepime or 13C_{2}D_{3}-cefepime as an internal standard, which provides several advantages in the accurate quantitation of drug levels in biological samples such as faster run time, improvement in intra-injection reproducibility, reduction of matrix effects and better sensitivity.

**Chromatography and detection**

Reverse phase chromatography was utilized for reported methods to separate cefepime from other sample molecules in the biological sample extract by partitioning between the mobile
### Table 1. Summary validation of various LC-MS/MS methods

<table>
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<td>1.</td>
<td><strong>Matrix:</strong> 100 μL of human serum&lt;br&gt;<strong>Extraction:</strong> protein precipitation with methanol: methyl-tert-butyl ether (90/10, v/v)&lt;br&gt;<strong>Internal standard:</strong> 30 μg/mL of 13C2D3-cefepime</td>
<td>Waters 2795 Alliance HT HPLC coupled to Waters Quattro micro API Tandem Quadrupole system&lt;br&gt;<strong>Column:</strong> Fortis C8 (100 × 2.1 mm, 3 µm) maintained at 30 °C.&lt;br&gt;<strong>Mobile phases:</strong> A – 10 mM ammonium formate with 0.1% formic acid in water. B – methanol. Flow rate: 0.5 mL/min in a step dilution mode.&lt;br&gt;<strong>Injection volume:</strong> 15 μL.&lt;br&gt;<strong>MS detection:</strong> cefepime: m/z 481.0 [M + H]⁺ → 167.0 (quant) and m/z 481.0 [M + H]⁺ → 395.7 (qual)&lt;br&gt;<strong>IS (13C2D3-cefepime):</strong> m/z 485.1 [M + H]⁺ → 167.1 and m/z 485.1 [M + H]⁺ → 400.0&lt;br&gt;<strong>Retention time:</strong> 1.66 min for both cefepime and IS&lt;br&gt;<strong>Total run time:</strong> 4 min.</td>
<td><strong>Regression type:</strong> linear fit with weighing factor of 1/x&lt;br&gt;<strong>Calibration range:</strong> 0.25–200 μg/mL. ((r^2 \geq 0.997).)&lt;br&gt;<strong>Absolute Recovery:</strong> 77.3 – 110.3%&lt;br&gt;<strong>Specificity:</strong> no interference peaks were observed at the retention time of cefepime and IS (n=5)&lt;br&gt;<strong>Carry-over:</strong> negligible&lt;br&gt;<strong>Dilution Integrity:</strong> upto 5x above HLOQ&lt;br&gt;<strong>Matrix Effect:</strong> No significant ion suppression&lt;br&gt;<strong>Intra- and inter-day Inaccuracy and imprecision:</strong> %CV: ≤6.8%. Relative bias: ≤8.4%. Relative root-mean-square-error (RSME): ≤8.4%.&lt;br&gt;<strong>Stability:</strong> serum samples stable at RT for 6 h, 24 h at 4 °C, two weeks at −20 °C, and 3 freeze-thaw cycles (−80 °C to RT).&lt;br&gt;<strong>Other drugs in the validated method:</strong> meropenem, ciprofloxacin, moxifloxacin, linezolid and piperacillin.</td>
<td>The validated method can be used for cefepime TDM</td>
<td>Paal et al. [44].</td>
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<td>2.</td>
<td><strong>Matrix:</strong> 100 μL of human plasma&lt;br&gt;<strong>Extraction:</strong> protein precipitation with methanol with 0.1% formic acid&lt;br&gt;<strong>Internal standard:</strong> 100 μg/mL of 2D3-cefepime</td>
<td>Ultra HPLC Ultimate 3000 RSLC coupled to Q Exactive Focus&lt;br&gt;<strong>Column:</strong> Accucore C18 (100 × 2.1 mm, 2.6 μm) maintained at 40 °C.&lt;br&gt;<strong>Mobile phases:</strong> A – 2 mM ammonium formate with 0.1% formic acid in water. B – Acetonitrile with 0.1% formic acid.&lt;br&gt;<strong>Flow rate:</strong> 0.3 mL/min in a step dilution mode.&lt;br&gt;<strong>Injection volume:</strong> 5 μL.</td>
<td><strong>Regression type:</strong> linear fit with weighing factor of 1/x&lt;br&gt;<strong>Calibration range:</strong> 0.50–32 µg/mL. ((r^2 \geq 0.99).)&lt;br&gt;<strong>Precision (Intra-day):</strong> 0.8 - 10.6%.&lt;br&gt;<strong>Precision (Inter-day):</strong> 3.9 -10.6%.&lt;br&gt;<strong>Accuracy:</strong> 96.1 ± 2.5%&lt;br&gt;<strong>Absolute recovery:</strong> ~90%&lt;br&gt;<strong>Specificity:</strong> no interference peaks were observed at the retention time of cefepime and IS (n=6)&lt;br&gt;<strong>Carry-over:</strong> negligible</td>
<td>The validated accurate mass quantitation method can be used for cefepime TDM</td>
<td>Lefeuvre et al. [41].</td>
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Table 1 Cont’d. Summary validation of various LC-MS/MS methods

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<td>2.</td>
<td><strong>MS detection:</strong> Full scan in positive mode. Cefepime [M + 2H]^2+: m/z 241.06976 [M + H]^+ → 395.7 (qual) 3D3-cefepime [M + 2H]^2+: m/z 242.57918</td>
<td>Acquity UPLC coupled to Acquity TQD tandem quadrupole mass spectrometer</td>
<td>Dilution Integrity: upto 10x above HLOQ Matrix Effect: No significant ion suppression Stability: plasma samples stable for 48 h at RT, 48 h at 2–8 °C, 20 days at −20 °C, and 3 freeze-thaw cycles (−80 °C to RT). Other drugs in the validated method: amoxicillin, oxacillin, piperacillin, ticarcillin, cefotaxime, ceftazidime, ceftriaxone, ertapenem, imipenem, meropenem, lincosamide (clindamycin), ofloxacin and ciprofloxacin and tazobactam</td>
<td>The validated accurate mass quantitation method can be used for cefepime TDM</td>
<td>Lefeuvre et al. [41].</td>
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| Matrix: 100 μL of human plasma | Extraction: protein precipitation with methanol | Internal standard: 3.22 μg/mL of 3D3-cefepime | Regression type: quadratic fit with weighing factor of 1/x² Calibration range: 0.50–175 μg/mL Precision (%CV, Intraday): 2.8 – 9.2%. Precision (%CV, Interday): 5.7 – 12.2%. Relative bias (Intraday): 12.3 – 13.7%. Relative bias (Interday): 5.0 – 9.2% Absolute recovery: 77.5 – 84.8% Selectivity: no interference peaks were observed at the retention time of cefepime and IS in patient samples receiving other drugs Carry-over: none Dilution Integrity: upto 10x above HLOQ Matrix Effect: ion enhancement <15%. Stability: plasma samples stable for 3 days at 5±3 °C. Other drugs in the validated method: amoxicillin, ampicillin, cloxacillin, piperacillin, cefazidime, cefuroxime, aztreonam and meropenem | The validated UPLC-MS/MS method can be used for cefepime TDM | Ri-go-Bonnin et al. [42]. |
Table 1 Cont’d. Summary validation of various LC-MS/MS methods

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| 4.     | **Matrix:** 50 μL of human serum<br>**Extraction:** protein precipitation with acetonitrile<br>**Internal standard:** 4 μg/mL of $^{13}$C$_3$-cefepime | 2D-Waters UHPLC coupled to Xevo TQ-S tandem mass spectrometer<br>**Column I:** Online SPE (Oasis HLB, 30×2.1 mm, 20 μm)<br>**Mobile phases:** A1– 0.1% formic acid in water. B1 – methanol/acetonitrile (80/20, v/v).<br>**Flow rate:** 2.0 mL/min<br>**Analytical Column:** Acquity UPLC BEH Phenyl (100 × 2.1 mm, 1.7 μm) maintained at 50 °C.<br>**Mobile phases:** A2– 0.1% formic acid in water. B2 – methanol/acetonitrile (75/25, v/v). Flow rate: 0.3 mL/min<br>**Injection volume:** 7 μL<br>**MS detection:** cefepime: m/z 241 $[M + 2H]^2+$ → 227<br>**Retention time:** 2.21 min for both cefepime and IS<br>**Total run time:** 5.0 min. | **Regression:** linear fit with weighing factor of $1/x$
**Calibration range:** 0.13–50 μg/mL
**Precision (%)CV, Intraday):** 3.47 – 8.53%.
**Precision (%)CV, Interday):** 4.07 – 6.04%.
**%RMSE (Intraday):** 5.75 – 8.77%.
**%RMSE (Interday):** 5.01 – 5.47%.
**Process efficiency:** 90%
**Selectivity:** no interference peaks were observed at the retention time of cefepime and IS in patient samples receiving other drugs
**Carry-over:** negligible
**Matrix Effect:** none
**Stability:** plasma samples stable for 2 h at RT, 12 h at 4 °C and 3 months at -80 °C.
**Other drugs in the validated method:** piperacillin, tazobactam, meropenem, ciprofloxacin and linezolid. | The validated 2D_ UHPLC-MS/MS method is being used for cefepime TDM | Zander et.al. [45]. |
| 5.     | **Matrix:** 50 μL of human serum<br>**Extraction:** solid phase extraction with Oasis HLB cartridges (30 mg)<br>**Internal standard:** 10 μg/mL of ethylparaben (20 μL) | Waters 2695 HPLC coupled to Micromass QuattroMicro API triple quadrupole mass spectrometer<br>**Column:** Unison UK-C18 (50×2 mm, 3 μm reversed phase porous ODS) maintained at 30 °C.<br>**Mobile phases:** A– 10 mM ammonium formate with 0.1% formic acid in water. B – methanol with 0.1% formic acid.<br>**Flow rate:** 0.3 mL/min<br>**Injection volume:** 20 μL<br>**MS detection:** cefepime: m/z 480.9 $[M + H]^+$ → 85.9<br>Ethylparaben (IS): m/z 165.0 $[M - H]^- → 91.8$
**Retention time:** 3.21 min for both cefepime and IS<br>**Total run time:** 13.0 min. | **Regression:** linear fit
**Calibration range:** 0.10–50 μg/mL ($r^2 =0.99$)
**Precision (%)CV, Intraday):** 3.1 – 9.2%.
**Precision (%)CV, Interday):** 6.9 – 12.6%.
**Accuracy (Intraday):** 94.2 – 101%.
**Accuracy (Interday):** 92.2 – 110.7%.
**Absolute recovery:** 88.5 – 100.4%
**Carry-over:** negligible
**Matrix Effect:** ≤2.6%
**Other drugs in the validated method:** ampicillin, cefazolin, cefmetazole, cefotaxime, doripenem, meropenem, and piperacillin | The validated HPLC-MS/MS method can be used for cefepime TDM | Ohmori et.al. [43]. |
and stationary phases. Ammonium formate buffer or water with 0.1% formic acid was used as the aqueous mobile phase and methanol or acetonitrile with 0.1% formic acid was used as organic mobile phase. Nonpolar or hydrocarbon-like systems (C8, C18, Phenyl and F5) were used as the stationary phases. The majority of reported LC-MS/MS methods utilized tandem mass spectrometry for the detection and quantitation of cefepime, with the exception of Lefeuvre et al. [41]. Cefepime is an inner salt and readily ionizes in the positive mode to provide excellent response. Multiple reaction monitoring transitions used by various methods is summarized in Table 1. The most common products of cefepime fragmentation that were utilized for quantitation were m/z 481.0 \([\text{M + H}]^+\) $\rightarrow$ m/z 167 and 86.1 [42-44]. In some cases, doubly charged ion of cefepime m/z 241 [M + 2H]^2+ was used to monitor the product m/z 227 for quantitation [41,45].

**DISCUSSION**

Recently published LC-MS/MS assays for cefepime TDM are summarized in Table 1. Paal et al. [44], reported a HPLC-MS/MS method for quantification of cefepime along with five other antibiotics in human serum (Table 1). The method employs a simple sample cleanup by protein precipitation and analysis utilizing stable labeled internal standards. Method utilized separation with C8 reverse phase HPLC column followed by MS/MS detection with clinically relevant concentration ranges. The assay demonstrated excellent selectivity, linearity and dilution integrity. Analytes were stable for 6 h at room temperature, 24 h at 4 °C, and two weeks at -20 °C.

Lefeuvre et al. [41], reported an UHPLC-HRMS assay for the simultaneous quantitation of cefepime and 14 other antibiotics in human plasma (Table 1). Sample cleanup involved protein precipitation followed by separation with C18 reverse phase chromatography. High-resolution full scan data acquisition was utilized for detection. Cefepime was stable for 48 h at room temperature and 2 – 8 °C and 20 days at -20 °C. This UHPLC-HRMS assay has the advantage of using acquired data for the retrospective analysis of metabolites.

Rigo-Bannin et al. [42], developed and validated a UHPLC-MS/MS method for simultaneous quantitation of ten β-lactam antibiotics in human plasma (Table 1). Sample cleanup involved protein precipitation, dilution, C18 reverse phase chromatographic separation followed by tandem mass spectrometric detection. The method demonstrated good linearity, selectivity and robustness to be utilized for TDM analysis. Cefepime was stable for 3 days at room temperature and 5 ± 3 °C and 6 months at -75 ± 3 °C.

Zander et al. [45], reported a UHPLC-MS/MS method for analysis of eight β-lactam antibiotics in human serum (Table 1). Sample cleanup involved SPE followed by C18 reverse phase chromatographic separation and tandem mass spectrometric detection. The assay demonstrated to be robust and successfully employed for the analysis of clinical samples.

Methods reported by Paal et al. [44], and Rigo-Bannin et al. [42], involved simple sample cleanup, robust chromatographic separation and tandem mass spectrometric detection. While other methods used complex sample preparation, 2D-HPLC or HRMS detection. Method by Paal et al. [44] is robust for analysis of cefepime in human serum with an efficient sample preparation and LC-MS/MS analysis. While method by Rigo-Bannin et al. [42] is robust for analysis of cefepime in human plasma with simple sample cleanup and UPLC-MS/MS analysis.

Cefepime has limited stability in human plasma and serum at room temperature and the duration of acceptable stability varies from 3 h to 3 days at room temperature based on...
different reports (Table 1). However, the reason for these significant differences in the stability of cefepime in human plasma and serum at room temperature remains unresolved. It is critical to establish sufficient stability of cefepime during sample collection, storage, and processing to generate accurate quantitative bioanalytical results.

Clinical Applications and Future Directions
In the current era of increasing antimicrobial resistance, it is paramount that antimicrobial dosing is personalized to assure optimal exposures that will result in clinical cure while minimizing the selection of resistant organisms. Therapeutic drug monitoring allows clinicians to provide individualized, target-oriented dosing for β-lactam antibiotics, including cefepime. Empiric dosing strategies, based on population PK modeling and Monte Carlo simulations, are designed to maximize the probability of target attainment. However, inter- and intra-variability in drug clearance and the volume of distribution prevents the use of a one-size-fits all dosing approach. The availability of LC-MS/MS assays that provide rapid and accurate measurement of cefepime concentrations can aid clinical pharmacists and clinicians in efforts to confirm drug exposures in individual patients, as well as facilitate adjustment of dosing in real-time to achieve desired levels. This is especially important in critically ill patients who require precise drug exposures to maximize bacterial killing and minimize the risk of toxicity. For instance, in a patient with a documented Gram-negative infection, clinicians can utilize TDM to estimate \( \text{FT}>\text{MIC} \) utilizing a variety of methods (i.e. Bayesian dose adaptation, log-linear regression, etc.). These data can then inform dose adjustments that aim to maximize the probability of target attainment in that patient. As described above, a number of PD targets have been associated with improved effectiveness outcomes, with >60% \( \text{FT}>\text{MIC} \) being the minimum time-dependent goal. TDM also holds potential to provide exposures that limit the selection of antimicrobial resistant phenotypes, although this has been predominantly established in vitro [14].

TDM in infants and children is challenged by a desire to minimize blood sampling, restrict entry into vascular access devices (i.e. central venous catheters), and avoid painful procedures such as venipuncture. Microsampling techniques can improve the performance of pediatric PK studies [46-48], applying less invasive approaches to blood sampling and reducing the risks to the patient. The availability of validated cefepime assays that utilize as little as 50 µL of plasma or serum can facilitate the clinical application of microsampling techniques and promote TDM in patients where sampling is challenging. Furthermore, application of whole blood microsampling (10-20 µL) to quantify cefepime levels in pediatric patients will be a convenient and efficient approach for TDM. Recently, Barco et al. [49], developed and validated a volumetric absorptive microsampling (VAMS™) assay for four antibiotics in human whole blood. VAMS™ approach allowed for accurate quantitation of drugs with no significant influence of hematocrit values. However, to employ VAMS™ approach for clinical studies, optimal collection, drying, shipping, and storage conditions with minimal drug degradation needs to be established. In addition, utility of VAMS™ approaches for TDM require converting whole blood concentrations to plasma or serum concentrations, where previously established reference ranges are available. Further studies comparing VAMS™, venous blood, and plasma samples are required to assess any potential difference between capillary blood from finger pricks and venous blood.

ACKNOWLEDGEMENTS
This work was supported through cooperative agreements from the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services with the sites of the Collaborative Pediatric Critical Research Network. KJD is supported by the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health under Award Number K23HD091365.
ABBREVIATIONS
ACN, acetonitrile; APCI, atmospheric pressure chemical ionization; CV, coefficient of variation; ESI, electrospray ionization; IS, internal standard; F/T, freeze–thaw; HLΒ, hydrophilic-lipophilic based; HRMS, high resolution mass spectrometry; IS, internal standard; LC, liquid chromatography; MeOH, methanol; MRM, multiple reaction monitoring; ODS, octadecylsilyl; PK, pharmacokinetic(s); RSD, relative standard deviation; SPE, solid phase extraction; TBME, ter butyl methyl ether; UHPLC, ultra high performance liquid chromatography; UPLC, ultra performance liquid chromatography.

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