



Review

Assays for therapeutic drug monitoring of β -lactam antibiotics: A structured review



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ABSTRACT

In some patient groups, including critically ill patients, the pharmacokinetics of β -lactam antibiotics may be profoundly disturbed due to pathophysiological changes in distribution and elimination. Therapeutic drug monitoring (TDM) is a strategy that may help to optimise dosing. The aim of this review was to identify and analyse the published literature on the methods used for β -lactam quantification in TDM programmes. Sixteen reports described methods for the simultaneous determination of three or more β -lactam antibiotics in plasma/serum. Measurement of these antibiotics, due to low frequency of usage relative to some other tests, is generally limited to in-house chromatographic methods coupled to ultra-violet or mass spectrometric detection. Although many published methods state they are fit for TDM, they are inconvenient because of intensive sample preparation and/or long run times. Ideally, methods used for routine TDM should have a short turnaround time (fast run-time and fast sample preparation), a low limit of quantification and a sufficiently high upper limit of quantification. The published assays included a median of 6 analytes [interquartile range (IQR) 4–10], with meropenem and piperacillin being the most frequently measured β -lactam antibiotics. The median run time was 8 min (IQR 5.9–21.3 min). There is also a growing number of methods measuring free concentrations. An assay that measures antibiotics without any sample preparation would be the next step towards real-time monitoring; no such method is currently available.

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1. Introduction

Infection is a severe problem in many areas of medicine. Sepsis alone is the leading cause of mortality in non-cardiac intensive care units (ICUs), with up to 30% of patients dying within 1 month of diagnosis [1]. Timely and appropriate antibiotic therapy after source control is considered to be the mainstay of treatment [2]. Achieving adequate antibiotic exposure is equally important; however, because of pathophysiological changes in the pharmacokinetics of the drugs, optimal dosing remains very difficult [3,4].

β -Lactam antibiotics are the most commonly used antibiotics because of their broad spectrum of activity and wide therapeutic index. They exhibit time-dependent pharmacodynamics, meaning that the duration that the free antibiotic concentration exceeds the minimum inhibitory concentration (MIC) of the pathogen ($fT_{>MIC}$) determines the bactericidal effect. Subtherapeutic concentrations using standard dosing have been reported in many patients groups, in particular critically ill patients [5–13], which in turn may result in clinical failure as well as the development of antibiotic resistance. Toxicity of β -lactam antibiotics is less common, but is severe when it occurs, with seizures from high concentrations being reported previously [14–18].

A more individualised approach using therapeutic drug monitoring (TDM) with dosing adapted to the altered pharmacokinetics of the individual patient is likely to be a strategy that can help optimise dosing [19]. TDM is mostly used for drugs with a narrow therapeutic index (such as aminoglycosides and glycopeptides) to

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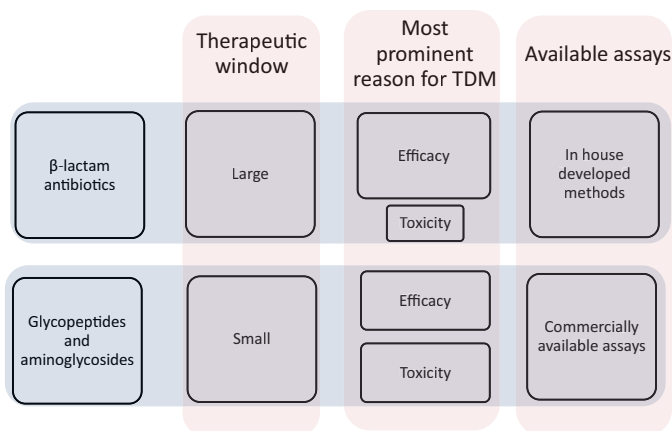


Fig. 1. Comparison of therapeutic drug monitoring (TDM) of β -lactam antibiotics with that of aminoglycosides and glycopeptides.

maximise efficacy and minimise toxicity, although only aminoglycosides have supportive published outcome data [20]. TDM of β -lactam antibiotics is a relatively new technique and although to date there is no evidence that this leads to improved clinical outcomes, it is increasing in popularity as a means to optimise dosing in difficult patient populations, mostly for reasons of efficacy [3,21].

However, unlike TDM of aminoglycosides and glycopeptides, no commercial assays such as immunoassays are available for routine monitoring of β -lactam antibiotics. A comparison of TDM of β -lactam antibiotics with that of aminoglycosides and glycopeptides is shown in Fig. 1.

The aim of this review was to identify and analyse the published literature on the methods used for β -lactam quantification in TDM programmes. In this review, we describe and compare the available methods to determine β -lactam antibiotics in plasma/serum and in other matrices, both for total and free concentrations.

2. Search strategy

2.1. Search terms

Data for the present review were identified using a literature search of PubMed from 1951 to January 2015 as well as references from within relevant papers and the extensive files of the authors. The search terms included: (((Beta-lactam OR penicillin OR cephalosporin OR carbapenem OR monobactam)) AND ('quantification' OR 'determination' OR 'assay' OR 'chromatographic' OR 'immunoassay')) AND ('dosage' OR 'patients' OR 'therapeutic drug monitoring' OR 'TDM' OR 'clinical samples').

2.2. Principles for the preferred method

Simultaneous analysis of three or more β -lactam antibiotics in serum/plasma was considered as an inclusion criterion for a method to be included in this review (one for alternative biological fluids, e.g. peritoneal fluid). The characteristics extracted from the included assays were run time, precision and accuracy of the method, calibration range, lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ). We also listed whether selectivity was tested in the case photometric detection was used, and whether the matrix effect was tested in the case of mass spectrometric (MS) detection.

3. Results

A total of 588 articles were reviewed for qualitative synthesis, of which 517 titles did not describe assay methods for quantification of β -lactam antibiotics but described general microbiological research, veterinary research or pharmaceutical research. Seventeen reports described methods for simultaneous determination of three or more β -lactam antibiotics in plasma/serum: 15 chromatographic methods [22–36] and 2 non-chromatographic methods [37,38]. Forty-two methods were found determining one or two β -lactam antibiotics, 12 of which determined at least one β -lactam antibiotic in other body fluids such as cerebrospinal fluid (CSF), ultrafiltrate or used an alternative sampling strategy such as dried blood spots [39–50]. Twelve papers reporting immunoassays were also found [51–62].

3.1. Methods to measure β -lactam antibiotic concentrations in plasma or serum

3.1.1. Chromatographic assays

There were 15 chromatographic methods (14 using reversed phase separation), coupled to ultraviolet (UV) ($n=9/15$) or to mass spectrometric (MS) detection ($n=6/15$). There appeared to be no difference whether plasma or serum was used. The characteristics of these methods are summarised in Table 1.

Three methods did not report data on specificity, and one method only reported the results of one blank sample [26,27,32,30]. Most of the manuscripts did not report on stability testing, although this is of major importance for β -lactam antibiotics, which in some cases are relatively unstable [63].

3.1.1.1. Analytes. The most frequently measured β -lactam antibiotic was meropenem, which was included in 14 of 15 methods, followed by piperacillin ($n=11$). The amount of analytes per run ranged between 3 and 12, with a median of 6 antibiotics [interquartile range (IQR) 4–10].

3.1.1.2. Calibration range. The ideal assay should have a LLOQ that is lower than the MIC of the likely causative pathogen. The LLOQ of most methods was indeed around MIC values for most pathogens (≤ 0.5 mg/L). Some methods reported a very high LLOQ, such as 2 mg/L or 5 mg/L for meropenem (higher than the MIC breakpoint for the least susceptible pathogens such as *Pseudomonas aeruginosa*), 5 mg/L for flucloxacillin or 10 mg/L for piperacillin [22,23,29]. Using a method with a high LLOQ may result in many trough samples measured as undetectable concentrations potentially leading to unnecessary dose adjustments for infections caused by lower MIC pathogens.

As both very high and very low concentration values can be expected when performing TDM in ICU patients where such a wide range of organ functions is possible, and turnaround time is strongly delayed if samples have to be re-analysed after dilution, it is important that the ULOQ is sufficiently high. An ULOQ of ≥ 100 mg/L should be preferred, especially for piperacillin, for which the most commonly used daily dosage is 12–16 g, resulting in high concentrations in many patients. However, 6 of 10 methods that quantified piperacillin reported an ULOQ of <100 mg/L [25,26,30,31,33,35]. One method reported the highest calibrator to be as low as 5 mg/L, which would not be considered convenient for routine TDM [31].

3.1.1.3. Run time. The median run time per sample was 8 min (IQR 5.9–21.3 min). However, for routine TDM shorter run times are desirable, as a batch with multiple calibrators and quality

Table 1Characteristics of the evaluated chromatographic methods for determination of β -lactam antibiotics in plasma.

Reference	No. of β -lactam antibiotics	Analytes	Column type	Detection	Total/free	Sample preparation	Run time (min)	LLOQ (mg/L)	ULOQ (mg/L)
[33]	10	Ampicillin, benzyl penicillin, cefazolin, ceftriaxone, cefalotin, dicloxacillin, ertapenem, flucloxacillin, meropenem, piperacillin	C18	UV at 210 nm for ampicillin, piperacillin, benzyl penicillin, flucloxacillin and dicloxacillin UV at 260 nm for ceftriaxone, cefazolin and cefalotin UV at 304 nm for meropenem and ertapenem	Free	Ultrafiltration and stabilisation	7	0.1	50
[24]	7	Amoxicillin, ampicillin, cefazolin, ceftazidime, cefuroxime, meropenem, piperacillin	C18	MS/MS	Total	Protein denaturation followed by back-extraction of acetonitrile	5.5	0.5	100
[36]	5	Amoxicillin, ceftazidime, cefuroxime, meropenem, piperacillin	C18	MS/MS	Total	Protein denaturation followed by dilution	2.5	1 (amoxicillin and piperacillin) 0.5 (ceftazidime, meropenem and cefuroxime)	150 for piperacillin 100 for amoxicillin, cefuroxime and ceftazidime 75 for meropenem
[31]	8	Amoxicillin, ampicillin, cefepime, ceftazidime, ceftriaxone, imipenem, meropenem, piperacillin	C18	MS/MS	Total	Protein denaturation followed by dilution	6	0.5	5
[25]	11	Amoxicillin, ampicillin, cefadroxil, cefazolin, cefepime, ceftazidime, cefuroxime, flucloxacillin, phenoxymethylpenicillin, piperacillin, meropenem	C18	MS/MS	Total	Solid phase extraction followed by dilution	4	0.05 for phenoxymethylpenicillin and cefepime 0.1 for meropenem 0.2 for cefuroxime and ceftazidime 0.25 for cefazolin 0.4 for amoxicillin 0.5 for flucloxacillin 0.6 for ampicillin and piperacillin 0.8 for cefadroxil 0.5	6 for phenoxymethylpenicillin and cefepime 9 for meropenem 20 for cefuroxime and ceftazidime 28 for cefazolin 50 for amoxicillin 58 for flucloxacillin 70 for ampicillin and piperacillin 91 for cefadroxil 100
[34]	4	Doripenem, ertapenem, imipenem, meropenem	5F-phenyl	UV at 295 nm	Total for doripenem, meropenem and imipenem Free for ertapenem	Stabilisation of the plasma sample, followed by protein denaturation and evaporation of the supernatant and reconstitution in buffer. Ultrafiltration for ertapenem	7		
[26]	5	Cefepime, ceftazidime, cefuroxime, meropenem, piperacillin	C8	256 nm for cefepime and ceftazidime 270 nm for cefuroxime 300 nm for meropenem 220 for piperacillin	Total	Solid phase extraction followed by evaporation and reconstitution in buffer	30	0.5 for ceftazidime, piperacillin and meropenem 1 for cefepime and cefuroxime	50

Table 1 (Continued)

Reference	No. of β -lactam antibiotics	Analytes	Column type	Detection	Total/free	Sample preparation	Run time (min)	LLOQ (mg/L)	ULOQ (mg/L)
[28]	3	Ertapenem, imipenem, meropenem	C8	UV at 298 nm	Total	Stabilisation of the plasma sample, protein denaturation, evaporation of the supernatant and reconstitution in buffer	25	0.5	80
[30]	8	Ampicillin, cefazolin, cefepime, cefmetazole, cefotaxime, doripenem, meropenem, piperacillin	C18	MS/MS	Total	Solid phase extraction followed by evaporation and reconstitution in buffer	13	0.005 for piperacillin 0.01 for cefepime 0.05 for cefmetazole and ampicillin 0.1 for cefazolin, cefotaxime and meropenem 0.5 for doripenem	50
[32]	3	Cefotaxime, cefradine, cefuroxime	C18	UV at 260 nm	Total	Solid phase extraction followed by evaporation to 2 mL	5	0.5 for cefuroxime 1 for cefotaxime 5 for cefradine	15 for cefuroxime 20 for cefotaxime 20 for cefradine
[29]	12	Amoxicillin, cefepime, cefotaxime, ceftazidime, ceftriaxone, cloxacillin, imipenem, meropenem, oxacillin, penicillin G, piperacillin, ticarcillin	C18	UV at 210 nm for cloxacillin, oxacillin, penicillin G, piperacillin and ticarcillin UV at 230 nm for amoxicillin, cefepime, ceftazidime, cefotaxime and ceftriaxone UV at 298 nm for imipenem and meropenem	Total	Protein denaturation using acetonitrile and dilution of the supernatant	20	2 for amoxicillin, cefepime, cefotaxime, ceftazidime, meropenem and imipenem 5 for ceftriaxone, cloxacillin, oxacillin, penicillin G, piperacillin and ticarcillin	250
[23]	12	Ampicillin, benzyl penicillin, cefazolin, ceftazidime, ceftriaxone, cefalotin, dicloxacillin, ertapenem, flucloxacillin, meropenem, piperacillin, ticarcillin	C18	UV at 210 nm for ampicillin, piperacillin, benzyl penicillin, flucloxacillin, ticarcillin and dicloxacillin 260 nm for ceftriaxone, cefazolin and cefalotin 304 nm for meropenem and ertapenem	Total	Protein denaturation using acetonitrile and back-extraction of the acetonitrile	25	5 for all analytes except for piperacillin and ticarcillin (10 mg/L)	250
[27]	5	Biapenem, doripenem, imipenem, meropenem, panipenem	C18	UV at 300 nm	Free	Dilution in mobile phase followed by ultrafiltration	9	0.04	25
[22]	6	Aztreonam, cefepime, ceftazidime, cefuroxime, meropenem, piperacillin	C18	UV at 240 nm for aztreonam and piperacillin UV at 260 nm for cefepime, ceftazidime, cefuroxime UV at 300 nm for meropenem	Total	Precipitation with methanol followed by evaporation of the supernatant and reconstitution in buffer	17	2	200
[35]	7	Benzyl penicillin, cefazolin, ceftazidime, ertapenem, flucloxacillin, meropenem, piperacillin	C18	MS/MS	Total	Protein denaturation using acetonitrile with 0.1% formic acid followed by dilution in water with 0.1% formic acid	7	0.1 for all analytes except flucloxacillin (0.25)	50 for meropenem, ertapenem, ceftazidime and cefazolin 25 for benzyl penicillin, piperacillin and flucloxacillin

LLOQ, lower limit of quantification; ULOQ, upper limit of quantification; UV, ultraviolet; MS/MS, tandem mass spectrometry.

control samples can require a high number of samples to be analysed consecutively.

3.1.1.4. Sample preparation. When developing an assay to determine the total concentration in plasma, a range of sample preparation procedures can be used, depending on the way the sample is pre-treated (protein precipitation using organic solvents or using solid phase extraction) and, depending on optional evaporation, the method used to concentrate the sample. Four methods used protein denaturation and subsequent dilution of the supernatant [29,31,35,36], only one used solid phase extraction and subsequent dilution [25], two methods used protein denaturation and back-extraction of acetonitrile [23,24], two methods used protein denaturation, evaporation of the supernatant and reconstitution of the residue [28,34], and three methods used solid phase extraction, evaporation of the supernatant and reconstitution of the residue [26,30,32]. Four assays were found that determined free concentrations, all of which used ultrafiltration [22,27,33,34].

3.1.1.5. Dried blood spots. Dried blood spots are a form of biosampling where a drop of blood is collected as a spot on a filter paper. It is most commonly used for the screening of metabolic disorders in neonates, but is increasingly being used for TDM purposes as well because of advantages including low volume of blood sampling, more convenient transportation, storage without special treatment, and enhanced analyte stability, which make it very attractive for TDM and/or pharmacokinetic studies [64].

We found one method that measured ertapenem from dried blood spots for TDM in neonates, in whom sampling of larger volumes is not practical, and two that measured piperacillin/tazobactam [39,50,65]. However, it must be noted that using this sampling strategy it is not possible to determine free concentrations, which may be a problem for highly protein-bound drugs such as ertapenem (ca. 90% protein bound). Moreover, the drying process after sampling takes ≥ 2 h, and dried blood spots may suffer from issues affecting the reliability of results including variations in the blood volume spotted, blood spot homogeneity and haematocrit concentrations. Variability in haematocrit (the volume percentage of red blood cells in blood) is a widely discussed challenge, which has an analytical and a physiological aspect [64]. When a fixed-diameter punch is taken from these spots, punches with a high haematocrit will contain a higher blood volume, which results in overestimation [64].

Compounds that do not enter erythrocytes will display low blood-to-plasma ratios (the ratio between the concentration of a compound measured in blood and the concentration measured in plasma). Hence, the presence of erythrocytes may be seen as a dilution of the plasma fraction of whole blood [64], and therefore bridging studies in which both dried blood spots and plasma samples are collected in order to evaluate the correlation between the concentrations are needed. A bridging study has been performed for piperacillin and tazobactam and found concentrations in dried blood spots to be on average 62% and 52% lower compared with plasma, suggesting that piperacillin and tazobactam do not partition into red blood cells. A large range in the dried blood spot-to-plasma ratios was observed [65]. Moreover, one of the biggest potential advantages of dried blood spots, namely stability, has been shown to be insufficient to allow for transportation at room temperature [50]. These findings may limit its use for TDM purposes.

3.1.2. Other methods to determine β -lactam antibiotics in blood

3.1.2.1. Thermal biosensing. Thermal biosensing detects the heat generated by enzymatic reactions, in this case the reaction of penicillinase with the β -lactam antibiotic [37]. This technique was used by Chen et al. [37] to determine concentrations of penicillin G,

penicillin V and ampicillin in whole blood and serum without any sample preparation.

Avoidance of sample preparation with this method reduces the turnaround time (time to which results are available) drastically and could even allow for point-of-care testing. However, there are issues that need to be resolved first. Chen et al. determined penicillin V, penicillin G and ampicillin, which are all susceptible to penicillinase, a requirement for the detection principle of this method. First, it is unclear whether this system would be able to monitor meropenem, which is one of the most widely prescribed β -lactam antibiotics, developed to be greatly resistant to β -lactamases. Second, other frequently used β -lactam antibiotics are co-formulated with a β -lactamase inhibitor, such as amoxicillin/clavulanic acid, piperacillin/tazobactam and ampicillin/sulbactam. Finally, similar to the dried blood spot analysis, bridging studies are needed to correlate the concentrations in whole blood to plasma concentrations.

3.1.2.2. Spectrofluorimetric determination. Some β -lactam antibiotics produce fluorescent degradation products. Therefore, the amount of light emitted by the degradation product after acid or alkaline degradation correlates with the initial concentration of the β -lactam antibiotic. Omar et al. developed a method to quantify seven cephalosporins that used spectrofluorimetric determination based on alkaline degradation [38]. This method is inexpensive as long as a luminescence spectrometer is available. However, a fairly complicated sample pre-treatment is needed with strict pH control and therefore it is not readily applicable for routine TDM [38].

3.1.2.3. Immunoassays. An immunoassay measures the concentration of an analyte in a solution using an antibody. Such immunoassays are commercially available for aminoglycosides and glycopeptides and are widely used for TDM of these compounds. Currently, no immunoassays are available for quantification of β -lactam antibiotics in human plasma. However, there are multiple assays available for trace analysis of antibiotics in milk and other food sources, with very fast analysis times [51–62]. Most of these immunoassays only give qualitative results, but some also give quantitative results, however in a concentration range that is far too low for TDM purposes as these tests are designed to quantify in the $\mu\text{g/L}$ range (which is necessary to detect antibiotic residues in these dietary products) whilst therapeutic values for these antibiotics in human plasma are 100–1000 times higher.

The advantage of using an immunoassay over the previously described chromatographic methods is that the equipment needed to perform an immunoassay is available in all clinical laboratories and should be easy to use. However, immunoassays can be troubled by interferences and cross-reactivity from similar compounds. Taking into account that patients may be switched from one antibiotic to another, the presence of the previous antibiotic may be problematic if the immunoassay is not sufficiently specific. Moreover, many different immunoassays, each with specific calibrators and controls, should be available in order to quantify all of the available β -lactam antibiotics.

3.1.2.4. Biosensors. Accurate quantification of small molecules using biosensors is emerging and appears very promising. Important applications of biosensing include glucose measurements in diabetic patients or detecting bacterial DNA using micro-arrays. A biosensor is made out of three parts: first, a biological sensor, such as an enzyme or a cell; and second, a transducer, which transduces the signal to the third part, the physicochemical detector, which for example has an increased fluorescence intensity when the ligand is added. Biosensors have been designed for some β -lactams yet they

have not been properly validated and compared with a reference method [66,67].

3.1.3. Free drug concentrations

Drugs are bound to serum proteins to varying degrees. The bound fraction is in equilibrium with the free (unbound) fraction. There is increasing interest in measuring this free concentration of antibiotics, given that the free concentration is responsible for bacterial killing as well as toxicity.

The two most relevant drug-binding serum proteins are albumin and α_1 -acid glycoprotein. It is often assumed that measuring free concentrations is only advisable for highly bound drugs ($\geq 95\%$ protein binding). Although this may be the case for healthy volunteers and general ward patients, many special patient groups (such as the critically ill, burn patients, undernourished patients or patients with nephrotic syndrome) frequently suffer from hypoalbuminaemia, which may significantly alter protein binding and, therefore, basic pharmacokinetic parameters of the free antibiotic such as volume of distribution and clearance [68,69].

Ultrafiltration is a simple method for measuring protein binding: the plasma sample is transferred to the upper chamber of a two-piece container separated by a molecular weight cut-off filter and when centrifuged separates the free fraction of drug into the lower chamber. However, ultrafiltration may be susceptible to non-specific drug adsorption to the container [70] and to variations in the experimental conditions such as pH, temperature and centrifugal force, which has been reported for vancomycin but also for the β -lactam antibiotics cefazolin and ertapenem [71–73]. Kratzer et al. report a free fraction for ertapenem of ca. 12.5% at 4 °C, but 20% when centrifuged at 37 °C [72]. Briscoe et al. were the first to report on a method to determine free concentrations of a range of β -lactam antibiotics using ultrafiltration [33]. Connor et al. reported free concentrations of piperacillin when centrifuged at 4 °C, which may not be the ideal temperature to accurately measure free concentrations [40].

Importantly, there are few comparative data between ultrafiltration and the technique that is considered the gold standard, equilibrium dialysis [74]. In equilibrium dialysis, two chambers are separated by a semipermeable membrane. These chambers are filled with serum/plasma and a buffer, respectively. This method needs a long time for equilibrium to be reached, which may pose a problem for some β -lactam antibiotics that are relatively unstable.

Wong et al. have compared the measured free concentration (using ultrafiltration) with the free concentration predicted from published protein binding values for seven β -lactam antibiotics using blood samples obtained from critically ill patients [75]. Significant differences between measured and predicted free drug concentrations were found only for highly protein-bound β -lactam antibiotics such as flucloxacillin (bias of 56.8% overprediction) and ceftriaxone (bias of 83.3% overprediction). No correlation between free and bound concentrations was found for these antibiotics, therefore direct measurement is considered essential for these drugs. For low to moderately protein bound antibiotics (such as piperacillin and meropenem), free concentrations appear to be predictable from the total concentrations [75].

3.2. Measuring β -lactam antibiotics in alternative matrices

Measuring β -lactam concentrations in other biological fluids may be beneficial as these fluids may be more closely related to the site of infection. Only methods determining β -lactam antibiotics in CSF and ultrafiltrate were found in this review, with no assays published for TDM in other fluids such as ascites fluid or epithelial lining fluid.

3.2.1. Cerebrospinal fluid

Nine articles were found reporting methods that determine β -lactam antibiotics in CSF. Two methods were found for meropenem [41,42], three for cefepime [43–45], two for ceftazidime [46,47], one for ceftriaxone [48] and one for cefotaxime [49]. Four of the nine methods used protein precipitation with acetonitrile as a sample preparation [43,47–49], four did not use any sample preparation [42,44–46] and one used ultrafiltration [41]. The detection method was liquid chromatography–ultraviolet (LC–UV) in five cases [41,43,47–49] and micellar electrokinetic capillary chromatography in three cases [42,44,46]. Micellar electrokinetic chromatography has the advantage of eliminating the need for sample preparation, which is advantageous to improve turnaround time. However, the migration time is quite long (10 min) and conditioning between runs is necessary [44–46].

3.2.2. Renal replacement therapy ultrafiltrate

Connor et al. investigated the relationship between free plasma concentrations of piperacillin and tazobactam and the concentrations in dialysate in patients treated with continuous venovenous dialysis in 50 samples from 19 patients and concluded that dialysate drug concentrations accurately predicted free plasma free drug concentrations ($R^2 = 0.91$ for piperacillin and 0.92 for tazobactam) [40]. However, the most evident deviation from unity was found in the lower-concentration piperacillin data, where dialysate concentrations underestimated plasma concentrations by as much as 50%. Therefore, it is unsure whether ultrafiltrate may replace plasma as a way to measure free concentrations although targeting higher concentrations with this technique may overcome this potential inadequacy.

4. General comments on setting up a method for therapeutic drug monitoring purposes

As routine TDM requires frequent runs (preferably once daily), the consequences of extensive sample preparation and long run times for the laboratory personnel and on equipment occupation are important. Therefore, when developing a method, minimising sample preparation is desirable while still retaining sufficient assay sensitivity. The ideal method should be able to measure both low (around MIC values of most commonly causative pathogens) as well as high analyte concentrations without dilution. Moreover, turnaround time should be kept to a minimum. As with all bioanalytical methods, the method should be thoroughly validated before it is used in clinical routine. Validation should include assessment of precision and accuracy, linearity, stability, interferences and matrix effect (in cases where mass spectrometry is used as the mechanism of detection). If one of the analytes is a β -lactam that is co-administered with a β -lactamase inhibitor (such as piperacillin/tazobactam or amoxicillin/clavulanic acid), it should be made sure that the β -lactamase inhibitor does not interfere. It is unclear whether or not the β -lactamase inhibitor should also be quantified during TDM, as there is currently no pre-defined target for the β -lactamase inhibitors and it is the β -lactam antibiotic that is responsible for the antibacterial effect.

The developed assay should include all commonly used antibiotics, both narrow- as well as broad-spectrum agents, as only applying TDM for the broad-spectrum antibiotics such as piperacillin and meropenem might lead to unnecessary switch from narrower-spectrum antibiotics to these broad-spectrum drugs whilst the reason for therapeutic failure might have been the altered pharmacokinetics of the narrower-spectrum antibiotics and a dose increase might have been enough to ensure optimal efficacy, and therefore TDM of narrower-spectrum drugs may potentially even spare the broad-spectrum and more potent

antibiotics. If highly protein-bound antibiotics are to be measured, free concentrations are preferred over total concentrations.

Pre-analytical stability must be guaranteed, as β -lactam antibiotics are generally considered to be quite unstable. However, this limited stability should not confound monitoring as these compounds are still stable for an adequate amount of time. The hospital staff must be aware that stability is limited and therefore samples should be sent to the laboratory as soon as the sample is taken, and the isolated plasma should be immediately frozen in the laboratory.

5. Future directions

The best of all cases would be a bedside sample collection device that requires only a small volume of whole blood but immobilises the proteinaceous and cellular component of the specimen to isolate the free fraction, thus yielding a sample requiring minimal preparation and providing maximal pharmacokinetic information; unfortunately, no such device is currently available.

Presently, TDM of β -lactam antibiotics requires the use of relatively expensive chromatographic techniques. As not all hospitals have this equipment available, easy transportation of the samples to a reference laboratory without the need for costly measures to prevent degradation would be most convenient. Therefore, more research into the utility of dried blood spots would be useful. Recently, two other promising sampling devices have been described. The first is the volumetric absorptive micro sampler, which consists of a polymeric tip and is designed to absorb a fixed volume of blood independent of haematocrit [76]. The second device is a paper collection disc bearing plasma from a fingertip drop of blood that can be air dried in 15 min and transported to the laboratory in a mailing envelope [77].

The cost of personnel to run chromatographic assays is also likely to be higher per sample than for the immunoassays used to measure aminoglycosides and glycopeptides. Therefore, another logical step would be to develop an easy-to-use assay that does not require expensive equipment or highly trained personnel. Immunoassays are available for the analysis of β -lactam antibiotics in food products, so technically it should be possible, as milk is an equally complex matrix as plasma. Therefore, more research in this field is also recommended. Liquid chromatography coupled to tandem mass spectrometric (LC–MS/MS) detection is a very powerful tool and has many advantages, such as enhanced specificity and the ability to simultaneously measure multiple analytes in highly complex biological matrices, but the adoption for clinical use has been limited because of instrument cost, expertise, training, quality assurance and standardisation [78]. However, progress is now being made in these areas with new instruments that allow analysis to be performed by general medical analysts, and the development of more user-friendly workstations with simplified sample preparation procedures. Finally, the commercial availability of reagent kits eliminates method development [78].

Last but not least, measuring free concentrations is an area that is in constant development. More research is needed about how to rapidly determine free concentrations to provide more accurate data.

6. Conclusion

Several methods have been developed and validated for TDM of β -lactam antibiotics. As quantification of these antibiotics is presently limited to in-house methods, most of the published methods use chromatographic separation coupled to UV or MS detection. There is currently no immunoassay available for TDM of β -lactam antibiotics.

Although many publications state the method under study is fit for TDM, some may not be considered highly practical because of intensive sample preparation and/or long assay run times. In order to develop a method for routine TDM, rapid sample preparation, short turnaround time, low LLOQ and sufficiently high ULOQ are vital. The antibiotics that require monitoring are dependent on the hospital usage. Indeed, most of the published assays monitored meropenem and piperacillin because they are widely used at many institutions.

There is a growing interest in measuring concentrations in other matrices, such as CSF, as well as measuring free concentrations. Ideally, a sample would be taken at bedside using a collection device that requires only a small volume of blood and can isolate the free fraction, which would yield a sample that requires minimal sample preparation and would be able to provide maximal pharmacokinetic information; unfortunately, no such device is currently available.

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