Review

The Role of Surface Enhanced Raman Scattering for Therapeutic Drug Monitoring of Antimicrobial Agents

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Abstract: The rapid quantification of antimicrobial agents is important for therapeutic drug monitoring (TDM), enabling personalized dosing schemes in critically ill patients. Highly sophisticated TDM technology is becoming available, but its implementation in hospitals is still limited. Among the various proposed techniques, surface-enhanced Raman scattering (SERS) stands out as one of the more interesting due to its extremely high sensitivity, rapidity, and fingerprinting capabilities. Here, we present a comprehensive review of various SERS-based novel approaches applied for direct and indirect detection and quantification of antibiotic, antifungal, and antituberculosis drugs in different matrices, particularly focusing on the challenges for successful exploitation of this technique in the development of assays for point-of-care tests.

Keywords: antibiotics; SERS; biofluids; Raman spectroscopy; TDM

1. Introduction

Severe infections are the most common cause of admission to intensive care units (ICUs), and a leading cause of death, affecting up to 1% of the global population annually, with increasing incidence and prevalence [1]. The incidence in Europe was reported as high as 270 new cases per 100,000 people for 2017, while more than 10 million people per year die from infection worldwide [2].

The emergence and spread of multidrug-resistant (MDR) pathogens that have acquired new resistance mechanisms, such as carbapenemase-producing enterobacteria or *Acinetobacter* spp., have made the treatment of infections in ICU patients increasingly challenging. At the same time, despite available guidelines, failure of antimicrobial treatment may also occur in ICU patients due to altered pharmacokinetic and pharmacodynamic properties of antimicrobial agents [3]. There is extensive evidence of sub-therapeutic exposure in critically ill patients from standard doses across different antimicrobials including aminoglycosides [4], beta-lactams [5], fluoroquinolones [6], glycopeptides [7], and azoles [8]. This can be a direct consequence of pharmacokinetic alterations coming from the complex pathophysiologic processes accompanying severe infection, including sepsis, shock, and organ failure [9].

To stop the growing number of MDR infections and reduce antimicrobial resistance (AMR), the World Health Organization approved a global action plan in 2017 [10]. Among the proposed solutions to maximize the success of antimicrobial therapy is the optimization of antibiotics use with the implementation of therapeutic drug monitoring (TDM) with the use of mathematical models to obtain a personalized dosing [11].

The basic principle of TDM is that the effects of a drug better correlate with circulating concentrations than with administered doses. Therefore, TDM involves the quantification of drug concentrations in blood, or in other biological fluids that can be linked to blood concentrations, to improve patient treatment outcome while minimizing the adverse effects inherent in the treatment. The measured drug concentration is thus used to adjust the drug dosing regimen by targeting a predefined concentration or exposure interval, called a therapeutic range. Ideally, individualized dosing strategies should account for individual pharmacokinetic (PK) changes and pathogen susceptibility [12].

Regarding the management of severe infections in ICU, the level of evidence of the benefits associated with the TDM of several antimicrobial agents is strong, with increasing interest [7,9]. TDM was traditionally adopted in clinical routine to prevent adverse toxicity effects, mainly for glycopeptides (vancomycin) and aminoglycosides (gentamycin, amikacin, and tobramycin) [3]. These antibiotics have a narrow therapeutic index, meaning that the window for either safety or toxicity is exceptionally small, and therapeutic ranges of blood concentrations have been defined. More recently, TDM of antimicrobial drugs slowly rose in broader use to guard against clinically inadequate concentrations and minimize the risk of the development of resistance, embracing diverse antibiotic classes including beta-lactams [13], antituberculosis [14], and antifungal drugs [8].

To adequately implement TDM, a range of bioanalytical techniques, such as immunoassays and single or mass-coupled chromatographic methods, are available. However, many of these methods are expensive with long turnaround times requiring specialized laboratories and trained personnel, thus limiting their use in hospitals in most underdeveloped countries. In this context, new advances in sensing technologies offer a huge opportunity to overcome these limitations. In recent years, surface enhanced Raman scattering (SERS) spectroscopy has grown dramatically with the development of nanosensors with engineered surface properties. SERS is a phenomenon associated with the amplification of the Raman signals of analytes located near the surface of nanostructured metallic substrates [15]. The growing availability of reliable commercial SERS substrates, coupled with relatively cheap portable Raman spectrometers, is helping SERS to emerge as a new innovative solution for the measurement of drugs in body fluids (especially in plasma, serum, and urine) [16–19], offering a high specific, sensitive, and low-cost technology that can be potentially brought to the patient's bedside and easily operated by doctors or health personnel.

In this review, after illustrating the peculiarities of TDM of antimicrobials, we summarize current trends in the use of SERS for the quantification of antibiotic, antifungal, and antituberculosis drugs in different matrices. We particularly focused on the challenges for successful exploitation of this technique in the development of assays for TDM, including synthesis and use of various forms of nanostructures as SERS substrates, the coupling with recognition strategies such as molecularly imprinted polymers or monoclonal antibodies, as well as the use of chemometrics and machine learning. A secondary objective of this review is to highlight some antimicrobial agents that would greatly benefit from SERS-based methods that could be carried out at the point of care.

2. Therapeutic Drug Monitoring of Antimicrobial Agents

TDM of antimicrobials can be figured as a dynamic three-step process: (a) quantification of drug concentration in plasma (or other biofluids), (b) interpretation of the results, and (c) dosage adaptation, if needed.

First of all, the reliability of the entire process is strongly related to the specificity and sensitivity of the employed method of analysis. As with any test in a highly regulated environment, such as hospitals, these methods should ideally be fast, fit-for-purpose, accurate, precise, selective, stable, and sensitive [20]. There are, however, also other requirements with respect to antimicrobial measurements. One problem is the very short half-life of antibiotics, which is usually due to either their degradation after sampling and/or their inherent chemical instability. Therefore, the preanalytical phase (sample preparation) should be reliable and as easy as possible, but also unaffecting drugs with

such low stabilities. Results should be available promptly, since turnaround times higher than 24 h should not be considered adequate for ICU requirements [12].

TDM measurements must be performed at the pharmacokinetic steady state and at the "trough" time point, i.e., at the end of the dosing interval (otherwise, special calculations are needed to estimate accumulation and elimination). As a rule of thumb, under regular dosage (intermittent administration or continuous infusion) and stable elimination mechanisms (renal excretion, hepatic metabolism), a stable steady state is reached after three to five drug elimination half-lives (Figure 1A). It is thus important to perform sampling at standardized time points after antimicrobial administration. However, defining the best sampling time may be challenging since the prescription strategy often relies on repeated administration. This underlines the need for well-documented, written protocols for managing TDM [21].
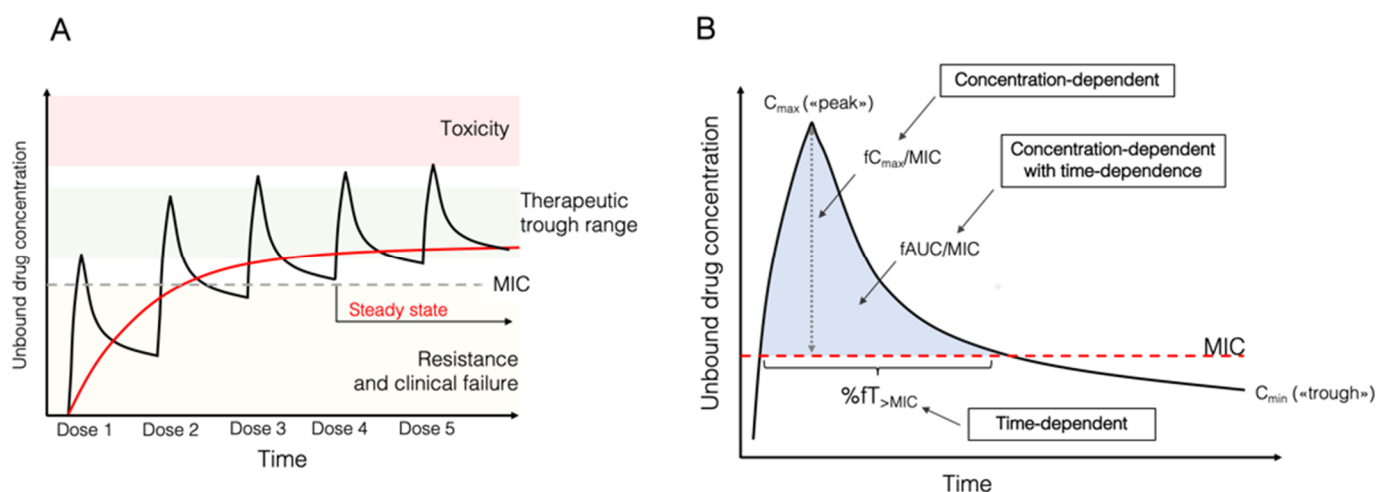

Figure 1. (**A**) Antimicrobial concentration–time curves. (**B**) Pharmacokinetic and pharmacodynamic parameters of antimicrobials at steady state. $\%fT_{>MIC}$, the percentage of time for which a drug's concentration remains above the minimum inhibitory concentration (MIC) for a dosing period; C_{max}/MIC, the ratio of the maximum antimicrobial concentration (C_{max}) to MIC; fAUC/MIC, the ratio of the area under the concentration–time curve during a 24 h time period ($AUC_{0–24\,h}$) to MIC.

After determining the antimicrobial concentration, the physicians can interpret the result and adjust the dosing regimen for the patient, through dosing adaptation models based on the pharmacokinetic (PK) characteristics of the drug of interest. However, the PK of many antimicrobials shows huge inter-individual variability and is largely unpredictable in ICU patients [22]. The exact mechanisms of this variability are not fully elucidated, but some of the following have been suggested: slower and variable gastrointestinal absorption (e.g., ciprofloxacin), increased volume of distribution (e.g., hydrophilic drugs such as aminoglycosides and beta-lactams), increased hepatic drug metabolism (e.g., sulfamethoxazole), and increased renal clearance (e.g., aminoglycosides, cephalosporins) [9]. The quality of the PK models can thus determine how successful the dose adjustment may be and must be validated with respect to the analytical technique.

In conclusion, the success of the entire TDM process depends not only on the measurement accuracy of drug concentrations, but also on adequate sampling, the quality of the dose adaptation model, and the extent of adjustments made to include the unique pathophysiological state of the individual.

2.1. Antimicrobial Quantification Methods

Most of the analytical platforms commonly used for TDM in clinical practice rely on many different immunoassays, or on separation techniques coupled with mass spectrometry (MS). The fundamentals and applications of these platforms will not be discussed in this

review but have been extensively reported elsewhere [23–25]. A brief summary is reported in Table 1.

Table 1. Comparison of different technologies for determining antimicrobials levels in biofluids.

Method	Multianalyte	Pros	Cons	Cost/Analysis
Immunoassay	No	Commercially available for most AM; simple to perform; direct from primary tube	Low accuracy; interferences from other drugs; may measure combined activity of parent and metabolites; time-consuming calibration.	High
LC-MS/MS	Yes	High sensitivity and accuracy	Initial costs; complexity of technology; not widely available; time consuming.	Medium
HPLC with UV/DAD detection	Yes	Widespread technology; commercially available assays; high accuracy	Manual sample preparation; subject to interference from various substances; run times may be slow.	Low
SERS	Yes	No need for sample preparation; fast measurement; ready for POC-T	Very often high RSD of the SERS substrates; method optimization needed for each drug.	Low

HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography combined with tandem mass spectrometry; POC-T, point-of-care testing.

Traditionally, immunoassays are *routine methods* used to provide the highest speed and ease of operation due to a high degree of automation. They are based on the detection of a drug via binding to drug-specific antibodies. The turnaround time is short and the cost per test is generally low. For these reasons, there are many commercially available assays for the determination of some antimicrobials requiring TDM. Some examples include QMS® Gentamicin (GENT), ARK™ Linezolid, Monoclonal Antibody (mAb) Penicillin (P2B9), QMS® Tobramycin (TOBRA), and QMS® Vancomycin. However, the low accuracy (acceptance range within ±20–25% of the nominal values at each concentration level for EMA of FDA approval [26]) of the results can be misleading. The antibodies used in the assay can provoke cross-reactivity with endogenous human antibodies and other biofluid components such as hemoglobin, drug metabolites, or structurally similar drugs, leading to falsely higher values [23]. Moreover, standardization and calibration of this type of testing are not always easy, thus requiring the expertise of trained laboratory personnel.

In contrast, *reference methods* such as liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) might be analytically more robust and accurate than immunoassays [27], and present less/no cross-reactivity, as reflected in the less stringent acceptance criteria for a successful validation for immunoassays with respect to LC-based techniques [26]. LC-MS/MS methods have been proposed for the quantification of several antimicrobials, including penicillins, carbapenems, macrolides, glycopeptides, and daptomycin. Despite their high specificity, interferences from the biological matrix may lead to falsely low or high results. Furthermore, the analysis is time-consuming and labor-intensive, and the throughput is lower than that of the conventional immunoassay platforms. Moreover, due to high initial investment and maintenance costs, facility requirements, and the need for highly trained analytical personnel, the implementation of LC-MS/MS methods is often not feasible in smaller and less-resourced hospitals. To mitigate the disadvantages of LC-MS/MS, high-performance liquid chromatography (HPLC) methods are often employed in combination with simpler detectors such as ultra-violet (UV) or diode-array detection (DAD). In such cases, however, analytes with similar retention times may interfere with the detection and overlapping peaks can be observed, especially in presence of unknown components in complex sample matrices.

2.2. Pharmacokinetic and Pharmacodynamic Indices of Antimicrobial Agents

Pharmacokinetic (PK) indices describe the time-dependent concentration course of the antimicrobial agent. The most important PK indices include the area under the plasma concentration–time curve ($AUC_{0-24\,h}$), the peak plasma concentration (C_{max}), and the trough (or residual) concentration (C_{min}), i.e., the concentration just before the next dose (practically about 5–10 min before it), as depicted in Figure 1B. Pharmacodynamics (PD) refers to the relationship between the antimicrobial concentration and the observed effect on the target pathogen, assessed in vitro under standardized conditions through a static and imperfect readout such as the minimal inhibitory concentration (MIC) [9]. MICs for TDM can be defined by various strategies [28], and indications on how to properly use it in TDM can be found elsewhere [29].

According to the differences in the dose–response relationships, antimicrobials can be broadly classified in one or more of the following PK/PD categories (Figure 2), describing antimicrobial exposure required for bacterial stasis or killing:

1. Time-dependent agents. The antimicrobial effect is dependent on the cumulative percentage of time over a 24 h period that the free (unbound) antimicrobial concentration surpasses the MIC ($fT\%_{>MIC}$). For these antimicrobials, the longer the concentration remains at serum levels above the MIC, the greater the effectiveness. Increasing their concentration any more than three or four times that of the MIC does not ultimately make a difference. Typical examples are beta-lactams.

2. Concentration-dependent agents. The antimicrobial effect is dependent on the peak concentration in a dosing interval divided by the MIC (C_{max}/MIC), with different target levels, usually $C_{max}/MIC > 8\text{–}10$. These antimicrobials would achieve optimal effect with higher immediate serum concentrations, and extending the time after which the concentration remains above the MIC would have little effect. Typical examples are aminoglycosides and daptomycin.

3. Concentration-dependent with time-dependent agents. The effect is defined by the $AUC_{0-24\,h}$ over a 24 h period divided by the MIC ($AUC_{0-24\,h}/MIC$). This ratio indicates the importance of both time and concentration for optimal effect. Examples are fluoroquinolones, tigecycline, linezolid, and glycopeptides. Specific targets vary according to the antimicrobial.
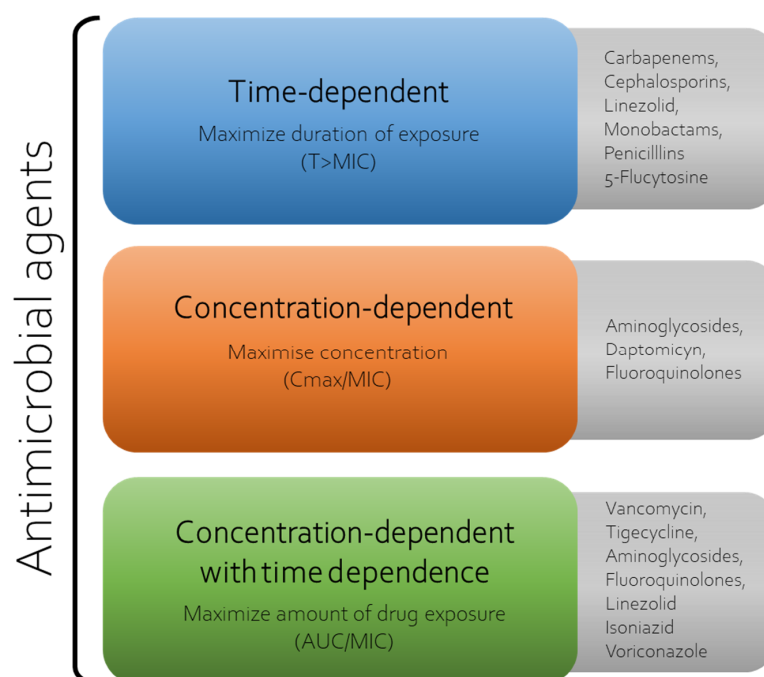

Figure 2. PK/PD classification of most common antimicrobial agents in intensive care units.

3. Surface Enhanced Raman Scattering and Therapeutic Drug Monitoring

As implicitly explained by the name, SERS is an analytical method based on the enhancement of the Raman scattering by nanostructured metal surfaces (generally called "SERS substrates", commonly made of gold or silver) with exceptional optical characteristics [30–32]. The information present in a vibrational spectrum, such as that obtained with Raman spectroscopy by analyzing the inelastic scattering of a laser light by a molecule, is strictly dependent on molecular structure, and thus it is often seen as a kind of "molecular fingerprint" of the analyte. Such information is exploited by analytical scientists to detect, identify, or even quantify molecular targets. Since Raman scattering is an intrinsic weak effect, however, the real breakthrough of SERS is the possibility of using Raman spectroscopy to investigate aqueous solutions with analytes in low concentration, i.e., µM to nM regime. SERS is thus considered both specific (because it has the "fingerprinting" capabilities of a vibrational spectroscopy) and sensitive (down to even a "single-molecule" detection regime by applying highly optimized measurement conditions and additionally the resonance Raman effect [33]).

The reasons why Raman scattering is amplified by several orders of magnitude are still investigated, but the general consensus settled for two mechanisms: a dominant, ever present "electromagnetic" mechanism relying on the plasmonic properties of the substrate, and another mechanism relying on the formation of a chemical bond between the analyte and the metal. Details of SERS theory can be found elsewhere [34,35], but the most important mechanism of enhancement (i.e., the electromagnetic) can be qualitatively described as the huge increase in the local electromagnetic field intensity at the metal substrate due to the collective oscillations of its surface electrons (i.e., plasmon polariton modes) caused by the incident light. While no direct chemical interaction between the analyte and the substrate is required for this mechanism to work, the amplification of the field is a local effect, so SERS requires nonetheless a close proximity of the analyte to the metal. The need for an adsorption or any kind of stable interaction between the analyte and the metal surface is a defining, essential feature of SERS: it sets both limits and advantages of this technique.

Since the electromagnetic mechanism is generally considered the most important one, SERS substrates are defined by their plasmonic properties. The ever-increasing, huge variety of SERS substrates, spanning from metal nanoparticles with different sizes and shapes to nanostructured surfaces with regularly spaced nano-sized features, is fully addressed elsewhere [36,37]. For the sake of simplicity, substrates could be roughly classified depending on the chemical nature of the metal (e.g., Au, Ag) or on their "state" (colloidal or solid). Colloidal aqueous dispersions of Au and Ag nanoparticles are among the most widely used substrates, since they are relatively easy to prepare with commonly available equipment upon chemical reduction of metal salts. Since the localized surface plasmon polaritons responsible for the electromagnetic mechanism of SERS are only excited at specific wavelengths, the laser wavelength used with a substrate is important to achieve the enhancement of the Raman signal. It is of utmost importance to realize that there are no universal, all-purposes SERS substrates, and that different substrates are suited for different applications.

In general, two different detection strategies can be used for SERS analysis: direct and indirect detection. In the direct detection, also called "label-free" detection, the SERS bands observed are due to the vibrational modes of the analyte itself, which is directly adsorbed on the substrate. The direct detection strategy is the most straightforward, but it relies on the spontaneous affinity of the analyte for the metal surface of the substrate. Heterocyclic aromatic compounds and thiols, for instance, do have such an affinity for Au and Ag, and can be usually detected with a label-free approach. Conversely, in the indirect detection, the SERS bands observed are due to the vibrational modes of a "Raman label" (also called "Raman reporter"), i.e., a molecule that is known to yield an intense SERS spectrum that is supposed to be associated with the analyte and the substrate. The indirect detection is more flexible, and can virtually be applied to any analyte, but it relies on an efficient recognition

element (e.g., an antibody) linking the analyte to the Raman label on the substrate of choice. This approach is often used to develop so-called "SERS immunoassays" [38,39].

Clinical Applications of SERS, including TDM, Quantitative SERS

With its sensitivity and specificity, SERS has been the object of intense research activity aimed at developing various bioanalytical and clinical applications, ranging from disease diagnosis and prognosis to identification of pathogens. The many biomedical SERS applications have been recently reviewed elsewhere [40–42]. For clinical applications, the availability of compact, portable, fast, and easy-to-use Raman instruments is particularly attractive to develop so-called "point-of-care" clinical applications, to be used at the patient's bedside by non-specialized operators.

These characteristics qualify SERS as an excellent candidate to develop TDM applications [16]. In particular, SERS methods for TDM based on both direct and indirect detection strategies aim at avoiding complex, time-consuming sample pre-treatment or separation steps, which are often required by other analytical approaches.

A major challenge for the application of SERS to TDM is the interference of the biofluids matrix. Biofluids are complex mixtures of thousands of biomolecules at varying concentrations, competing with the target drug for the adsorption sites at the metal surface of the substrate. This competition is less critical for indirect detection strategies, since the recognition elements bind to the drug of interest with a certain selectivity. However, for approaches involving a direct detection with SERS, this interference of the biological matrix can be a major obstacle to the target detection, more so considering the inter-individual variability of biofluids composition. The use of multivariate analysis methods may mitigate this problem, as the signal of the drug of interest can be "extracted" from that of the matrix. Biofluids commonly used in TDM such as plasma and serum are also rich in proteins, which are known to readily adsorb on metals, causing a so-called "surface fouling" effect. Moreover, these proteins often bind to a significant fraction of the drug of interest, hampering a direct interaction of the drug with the metal surface. Where necessary, rapid pre-processing steps involving sample deproteinization are often adopted before SERS detection to solve such protein-related issues.

Another major challenge for most SERS-based TDM applications is that a quantification of the analyte drug is needed. This is considered a challenge mainly because of both intra- and inter-substrate (i.e., batch-to-batch) repeatability issues with most SERS substrates, causing uncontrolled variability in the intensity of SERS bands. These issues originate from the fact that most substrates have a random density and distribution of hot spots (i.e., nano-sized gaps between metal nanostructures where the electromagnetic enhancement is particularly intense), since these two aspects are difficult to control during the fabrication process. Increasing the number of measurements and applying a proper statistical approach might mitigate this problem, although at the cost of increasing the time of analysis. The use of internal standards, although not devoid of problems, is also considered a viable solution for this repeatability issue [43–45]. Even when measures are taken to quantify or compensate for substrate-related variability, the lack of a standardized approach in reporting SERS results makes difficult the comparison between methods developed by different labs [46].

4. SERS Applications for TDM of Antimicrobial Agents

Research has been undertaken to develop SERS-based technology suitable for the point-of-care detection of antibiotics [30]. A list of relevant examples is enumerated in Table 2. In addition, a selection of the most relevant developments is discussed in more detail herein. The development of SERS-based methods for antimicrobial detection and quantification initially began as a method to determine contaminants in food and the environment [47,48]; antibiotic detection in milk is one of the more commonly reported areas, due to contamination resulting from animals undergoing therapy. Although studies that provided data on real biofluids have been prioritized for this review, it should be noted

that the goal of a simple and effective method for analyzing antibiotics in complex matrices, such as milk or honey, is analogous to TDM, and therefore, there is much to be gained in learning from the approaches pursued in those assays.

Table 2. A summary of the selected SERS-based application for the detection and quantification of antimicrobial agents in different matrices.

Family	Analytes	Substrate	Laser	Matrix	LOD	LOQ	Ref
Aminoglycosides	Amikacin	cAg	532	water	na	na	[49]
	Neomycin	SERS-LFA	633	milk	0.37 pg/mL	na	[50]
	Tobramycin	Au-DTNB NPs	633	human serum	na	na	[51]
Antifungal	5-flucytosine	sAg	785	serum	12.1 μg/mL	na	[52]
	Voriconazole	cAu	785	plasma	na	na	[53]
AntiTB	Isoniazid	cAG	488	water	5 ng	na	[54]
Carbapenems	Meropenem	sAu	785	plasma	na	na	[55]
Cephalosporins	Cefazolin	cAg	638	urine	na	na	[56]
	Cefazolin	cCu	638	urine	8.8 μg/mL	90 ng/mL	[57]
	Cefoperazone	cAg	638	urine	na	na	[56]
	Cefoperazone	cCu	638	urine	36 μg/mL	1 pM	[57]
	Cefotaxime	cAg	638	urine	na	na	[56]
	Ceftazidime	sAg	785	water	na	na	[58]
	Ceftriaxone	CaCO3-Cu-NPs	633	water	5 μM	89 ng/mL	[59]
	Ceftriaxone	cCu	638	urine	7.5 μg/mL	19 μg/mL	[57]
	Ceftriaxone	cAg	638	urine	na	na	[56]
	Ceftriaxone	cAg	473	urine	0.4 μg/mL	45 μg/mL	[60]
	Cefuroxime	cAg	638	urine	na	na	[56]
Fluoroquinolones	Ciprofloxacin	Ag-TiO$_2$ NPs	633	water	70.8 pM	34 μg/mL	[61]
	Ciprofloxacin	SERS-LFA	633	milk	0.57 pg/mL	na	[50]
	Ciprofloxacin	cAg	514	water	0.1 μM	0.1 mM	[62]
	Ciprofloxacin	sAg	785	water	na	na	[63]
	Ciprofloxacin	Au NRs	633	water	0.01 ppm	92 μg/mL	[64]
	Ciprofloxacin	sAg	785	water	0.351 ppb	2 μg/mL	[65]
	Enoxacin	Ag-TiO2 NPs	633	water	315 pM	280 μg/mL	[61]
	Levofloxacin	cAg	532	urine	0.07 mM	na	[66]
	Levofloxacin	EC-SERS	785	synthetic urine	na	na	[67]
	Levofloxacin	Au@Ag NRs	633	water	0.37 ng/L	na	[68]
	Levofloxacin	cAu	633	mice blood	na	1 ppm	[69]
	Levofloxacin	optical fiber nano-probe	633	mice blood	na	na	[70]
	Moxifloxacin	sAg	514	water	100 nM	na	[71]
	Moxifloxacin	sAu	785	urine	0.085 μg/mL	na	[72]
	Ofloxacin	cAg	532	methanol	23.5 ng/mL	na	[73]
Macrolide	Erythromycin	cAg	532	water/ethanol	na	na	[74]
Monobactam	Aztreonam	cAg	633	water	na	na	[75]
Penicillins	Amoxicillin	sAg	785	water	2.4 nM	na	[76]
	Amoxicillin	cCu, Cu-GO	785	water	0.0157 μM	na	[77]
	Ampicillin	cAg	533	water	27 ng/mL	na	[78]
	Cloxacillin	sAu	780	methanol/acetic acid (9:1)	7.8 pmol	0.15 μg/mL	[79]
	Penicillin G	SiO2@Ag-NRs	532	water	0.001 nM	72.6 ng/mL	[80]
	Penicillin G	Ag-GO	785	water	0.3 nM	na	[81]
	Penicillin G	cAg	533	water	29 ng/mL	na	[78]

Table 2. *Cont.*

Family	Analytes	Substrate	Laser	Matrix	LOD	LOQ	Ref
Phenicols	Chloramphenicol	cAu	785	water	0.1 µg/mL	na	[82]
	Chloramphenicol	cAu	785	eye drops	0.01 µM	na	[83]
	Chloramphenicol	sAg	633	honey	4 ng/mL	na	[84]
Polymixins	Colistin	SERS-LFIA	785	milk	0.10 ng/mL	na	[85]
Sulfanilamides	Sulfadiazine	cAg	514	water	1 ng/mL	na	[86]
	Sulfamethoxazole	cAg	488	urine	1.7 µg/mL	na	[87]

cAg, silver colloidal nanoparticles; cAu, gold colloidal nanoparticles; cCu, colloidal copper nanoparticles; GO, graphene oxide; LFIA, lateral flow immunoassay; NRs, nanorods; sAu, gold solid substrate; sAg, silver solid substrate.

4.1. Time-Dependent Agents

Cephalosporins are the family of time-dependent antibiotics for which the most promising SERS data are available in studies explicitly aiming at TDM, mostly thanks to the works by Markina et al. [56,57,59,60]. The methods presented in these works, all based on a direct detection strategy, have LODs or LOQs in a biofluid (i.e., spiked urine) which are compatible with the requirements of TDM. All cephalosporins should be monitored for efficacy when used in critically ill patients, and cefepime should be monitored for both efficacy and toxicity [12]; however, due to the lack of any commercial assay, clinical laboratories must develop and validate their own assays. Urine is easily available without any invasive measures and is an analytical matrix much easier to handle than serum or plasma because it does not contain many proteins.

In particular, the most promising results were obtained for ceftriaxone, cefazolin, and cefoperazone using Cu nanoparticles with an excitation wavelength at 638 nm [57]. The use of Cu as metal, which likely has a stronger interaction with the carbonyl and carboxyl groups of the drug molecules, allowed avoiding a solid phase extraction step, which has been deemed necessary to remove interfering metabolites when Ag nanoparticles were used as substrates [56,57]. The main drawback of the Cu nanoparticles used, as reported by the authors, is their low chemical stability, requiring the use of freshly prepared batches for SERS analysis and thus limiting the versatility of the method.

With some exceptions, most SERS studies on other time-dependent antibiotics were performed in water, limiting their significance with respect to TDM applications in complex biological matrices. This is the case, for instance, of the antibiotics belonging to the penicillin family, for which several label-free SERS studies are available (see Table 2) in aqueous solutions. The reasons for the lack of SERS studies on penicillins on spiked biological fluids is that the interest for a TDM of these molecules is relatively recent (consensus on TDM-guided dosing is still lacking [5]), while research was focused on environmental or food contamination of these drugs. LODs and LOQs of SERS analysis for several penicillins are low enough to raise the interest for TDM applications, but these figures of merit are expected to worsen when interferences from biological matrix constituents come into play. Thus, further studies are needed to check the applicability of these methods on spiked biofluids. The only penicillin that has been quantified with label-free SERS from a biofluid (i.e., spiked pig plasma) is cloxacillin, by using a solid-phase extraction based on a molecular imprinted polymer, followed by a recovery step with a methanol/acetic acid mixture (Figure 3) [79]. This work by Ashley et al. is an example in which a label-free SERS detection is achieved by decreasing the complexity of the matrix by a pre-treatment step, but at the expense of an overall increase in measurement times, costs, and complexity.

Meropenem and sulfamethoxazole (two antibiotics of the carbapenem and sulfanilamide families, respectively) were also quantified by using label-free SERS methods in complex biological matrices [55,87]. In both studies, which explicitly aimed at TDM, the results were extremely promising. Muneer at al. quantified meropenem in human blood plasma down to a LOQ of 1 pM on gold nanostructures on nickel foams excited in the NIR, using a hand-held Raman spectrometer [55]. As in the case of some cephalosporins,

Markina et al. succeeded in quantifying sulfamethoxazole from spiked urine samples by relying on a case-specific sample pre-treatment to separate the target from interfering metabolites [87]. In the case of sulfamethoxazole, the pre-treatment step, involving liquid–liquid extraction, pH adjustment, and dilution, was considered important to achieve a reliable SERS quantification method, at least using Ag nanoparticles as substrate (Figure 4).
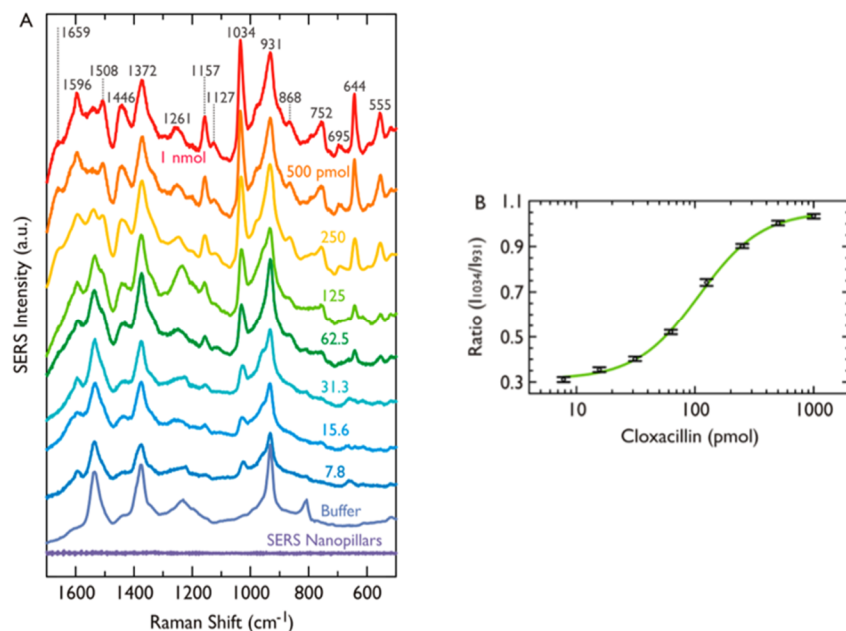

Figure 3. (**A**) SERS spectra for different amounts of cloxacillin deposited on the nanopillar substrate. SERS intensities were normalized to improve visual clarity. (**B**) The nonlinear calibration plot (*n* = 3). Each ratio of intensity was averaged over 121 spectra obtained in a 1 mm × 1 mm droplet footprint. Reproduced with permission from [79]. Copyright 2017 American Chemical Society.
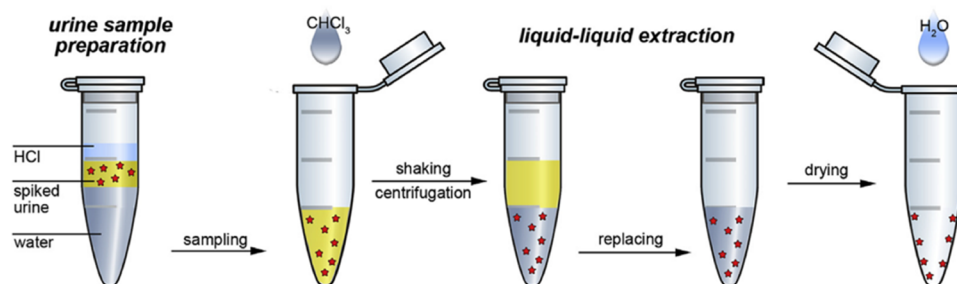

Figure 4. An example of urine sample preparation and LLE procedure. Reproduced with permission from [87]. Copyright 2022 Elsevier.

As far as the antifungals are concerned, only one study on the label-free SERS quantification of 5-flucytosine in spiked serum is available [52]. Even if routine TDM for this drug is neither recommended nor discouraged, the work by Berger et al. is particularly appealing since it directly measures the drug in undiluted serum, without any sample preprocessing. Data presented suggest that a passive vertical flow system constituting several nitrocellulose membranes is capable of "cleaning" the serum sample before adsorption on Ag nanoparticles deposited on paper, allowing the drug SERS bands to be observed (Figure 5).
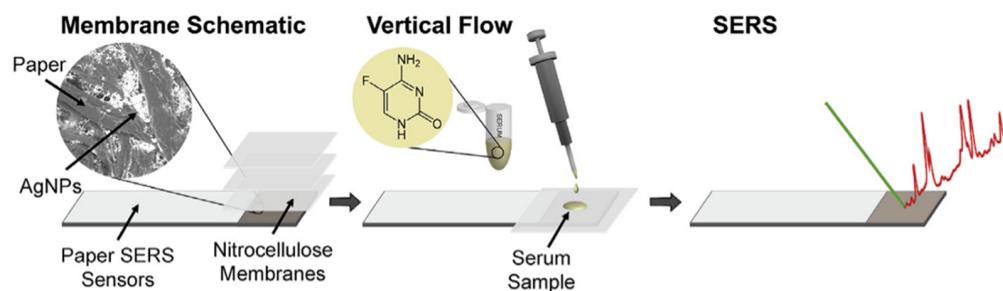
Figure 5. Setup, vertical flow, and detection schematic. Vertical flow using a microporous membrane scheme separates the flucytosine from the serum. SEM of the inkjet-printed AgNPs on cellulose paper is shown. The SERS signal is read from the paper SERS sensors. Reproduced with permission from [52]. Copyright 2017 Elsevier.

4.2. Concentration-Dependent Agents

TDM of aminoglycosides has been the standard of care in many hospitals since the 1970s as a means to reduce toxicity while still assuring adequate levels of these antibiotics for treatment of severe infections. McKeating et al. showed how SERS can be used in combination with localized surface plasmon resonance (LSPR) for the analysis of various aminoglycosides and performed an in-depth study of the feasibility of carrying out TDM of tobramycin in spiked human serum, down to 4 μM [51]. The study was conducted using a 633 nm laser to excite gold nanoparticles conjugated with 5,5′dithiobis-(2-nitrobenzoic acid), DTNB. Interestingly, the covering of the surface of the gold nanoparticles with DNTB, a common Raman reporter, was necessary to obtain an intense SERS spectrum directly related to the extent of aggregation provided by the aminoglycosides. Moreover, a dilution of the crude serum would be required for the analysis of real samples, due to the low sensitivity obtained.

Similarly, a SERS-based lateral flow immunosensing assay (LFIA) using DNTB-Au NPs has been proposed to identify colistin in milk. Colistin is a long-known cationic polypeptide antibiotic. Its use has recently resurged, assuming an important role as salvage therapy for otherwise untreatable infections in ICU. International consensus guidelines recommend TDM and adaptive feedback control for colistin, because drug dosage cannot be safely optimized using clinical observations and dosing algorithms alone, especially in the important early treatment period. The assay developed by Li et al. showed rapidity (assay time below 20 min), high accuracy (with recovery between 88% and 113%), and a satisfactory precision below 15%.

Liu et al. demonstrated the quantification of voriconazole from plasma samples using a portable Raman spectrometer (i-Raman Plus), relying on gold nanoparticles and chemometrics [53]. Considering the substantial and multifactorial variability in pharmacokinetics, the exposure-dependent occurrence of predominantly neurotoxic adverse effects, and significant relationships between exposure and outcome, TDM is recommended for adults and children by current international guidelines when voriconazole is used for prophylaxis or treatment of invasive fungal infections [8].

The feasibility of the SERS-based method was evaluated on five patient samples and validated using a standard HPLC method. Comparison of the voriconazole plasma concentrations, obtained using both methods, yielded a mean relative difference of 7%, indicating a good agreement. However, the authors of the work suggested that further studies using a much larger number of patient samples will be required to better assess the accuracy of the SERS-based method.

4.3. Concentration-Dependent Agents with Time Dependence

Fluoroquinolones exhibit concentration-dependent bacterial killing as serum concentrations increase to approximately 10 times the MIC, and also offer a post-antibiotic effect (PAE). However, there are few PK data in the literature for special patient populations,

especially in critically ill patients. As a result, recommended doses of fluoroquinolones, and in particular, those for ciprofloxacin for special patient populations, offer an extensive range, raising the risk for both under- and over-dosage. Moreover, multiple concentration measurements widely distributed over the dosing interval are required to compute the AUC_{0-24h}. Since more than 85% of the administered dose of levofloxacin is excreted into the urine, monitoring the clearance rate of levofloxacin could be adopted to reduce the burden of frequent sampling for both patient and personnel while providing an important way to access treatment effectiveness and reduce side effects.

The work by Shi et al. introduced a SERS-LFIA for the ultrasensitive detection of many fluoroquinolones in milk using AuNPs conjugated to 4-aminothiophenol (PATP, the Raman reporter molecule), and to monoclonal antibody against specific antibiotics [88]. The proposed method exhibited high sensitivity, specificity, and reliability, with a LOD of 0.57×10^{-3} ng/mL for ciprofloxacin.

More interestingly, a droplet-based microfluidic detection scheme in the form of lab-on-a-chip SERS (LOC-SERS) was applied for the SERS-based quantification of the levofloxacin in spiked human urine samples (Figure 6) [66]. Using silver nanoparticles as a method of enhancing the Raman signal, the levofloxacin spectra were elucidated from a microfluidic device at detection limits (LOD, 0.8 µM) and linear dynamic window (1–15 µM) within the target therapeutic range. Bindesri et al. reported the detection of levofloxacin from synthetic urine samples using the first fabric-based sensor combining electrochemistry (EC) and SERS [67]. Conductive inks and AgNPs paste were used to manufacture the EC-SERS sensor on a cotton blend fabric. Such wearable sensors could be included in clothing articles and facilitate continuous monitoring of a patient's health. In this study, the authors successfully tested their sensor for levofloxacin down to a concentration of 1 mM.
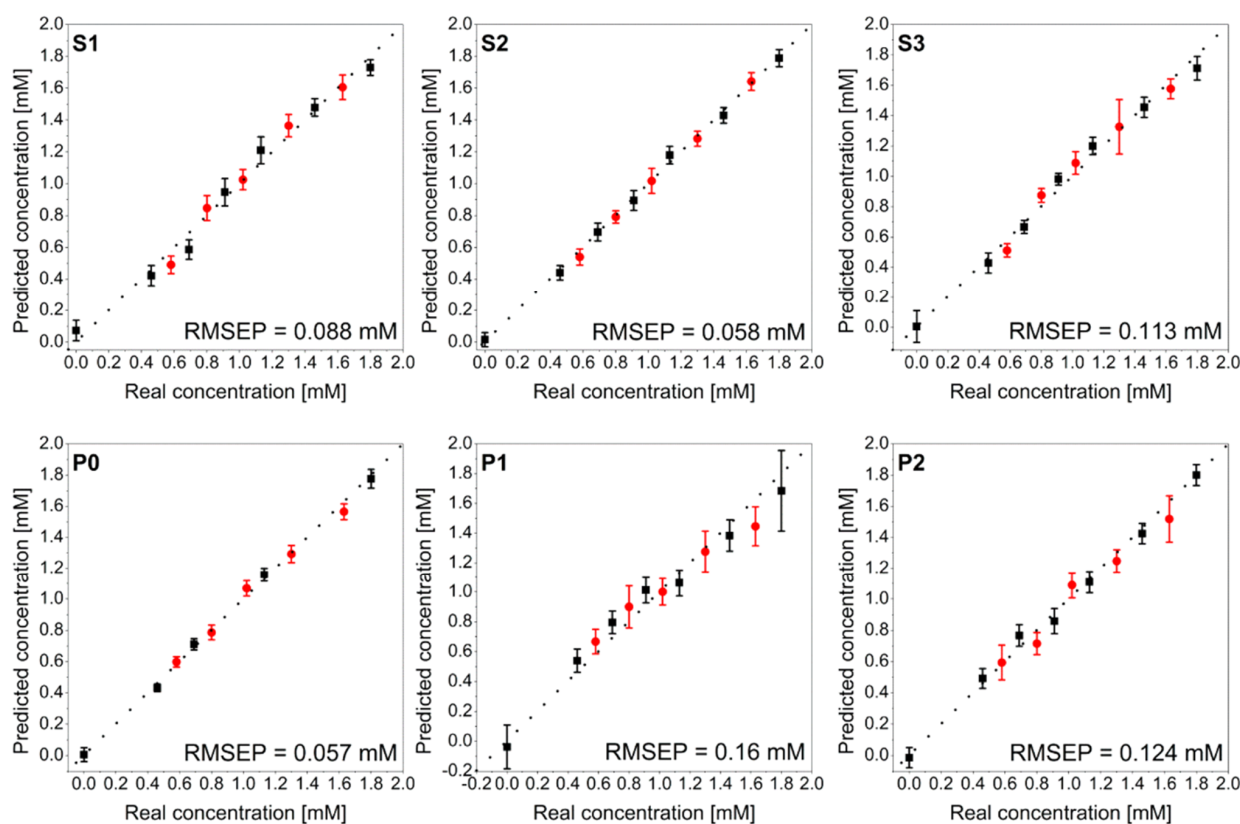

Figure 6. PLS prediction plots for spiked human urine samples (three individual, S1, S2 and S3, and three pooled urine samples, P0, P1 and P2) using seven concentrations for training (black scatter) and five (red scatter) for predicting. Reproduced with permission from [66]. Copyright 2016 American Chemical Society.

Moxifloxacin was also detected in urine by Mamian-Lopez and Poppi using nanostructured gold surfaces [72]. The standard addition method in conjunction with multivariate curve resolution–alternating least squares was applied to eliminate the matrix effect and to isolate the spectral contribution of the analyte. The low LOD (0.085 µg/mL) and LOQ (0.26 µg/mL) values indicated that the quantification can be accomplished in urine up to 24 h after the administration of a single 400 mg dose.

A secondary objective of this review is to highlight some antimicrobial agents that would greatly benefit from SERS-based methods that could be carried out at the point of care. The examples provided in Table 3 constitute a subset of potential applications from a list of the most monitored drugs. The development of SERS methods for clofazimine, ertapenem, gentamycin, linezolid, oxacillin, piperacillin, ticarcillin, tigecycline, and vancomycin would be of great interest.

Table 3. Availability of SERS-based methods for the TDM of most commonly used antimicrobial agents.

DRUG	Effective Concentrations (µM)	Toxic Concentrations (Cmin, µM)	Protein Bound Fraction (%)	TDM	SERS Method	Ref
Amikacin	7–14 (trough); 25–43 (peak)	>14 (trough); >55 (peak)	<10	required	+	[89]
Amoxicillin	86–172	>988	18	recommended for ICU patients	++	[90]
Ampicillin-sulbactam	23–106	>106	28	recommended for ICU patients	++	[91]
Aztreonam	207–376		56 (36–43)	recommended for ICU patients	+	[12]
Cefazolin	88–176		74–86	recommended for ICU patients	+++	[90]
Cefepime	10–73	>46	20	recommended for ICU patients	+++	[90]
Cefotaxime	65–132		35–45	recommended for ICU patients	+++	[90]
Ceftazidime	64–146		<10	recommended for ICU patients	++	[90]
Ceftriaxone	44–120		95	recommended for ICU patients	++	[90]
Chloramphenicol	31–77	>77	50–60	required	++	[92]
Ciprofloxacin	3–30	unclear	20–40	recommended for ICU patients	++	[12]
Clofazimine	1–4		99.99	recommended (TB)	nr	[93]
Cloxacillin	46–115		95	recommended for ICU patients	+	[12]
Colistin	unclear	>2	69–74	required with prolonged dosing	++	[94]
Daptomycin	<15	>15	90–93	neither recommended nor discouraged	nr	[95]
Ertapenem	11–21 (trough)		85–95 cd	recommended for ICU patients	nr	[90]
Fluconazole	>33–49 (trough)		11–12	neither recommended nor discouraged	nr	[12]
5-Flucytosine	194–387 (trough); 387–775 (peak)	>775	3–4	neither recommended nor discouraged	+++	[12]
Gentamycin	<4 (trough); 10–21 (peak)	>4 (trough); >25 (peak)	0–30	required	nr	[12]

Table 3. *Cont.*

DRUG	Effective Concentrations (µM)	Toxic Concentrations (Cmin, µM)	Protein Bound Fraction (%)	TDM	SERS Method	Ref
Isoniazid	22–51 (peak, 300 mg dose) *; 66–131 (peak, 900 mg dose) *	>146	<10–15	recommended	++	[93]
Itraconazole	0.7–1.4	<6	99.8	required	nr	[12]
Levofloxacin	0.83–36	unclear	24–38	recommended for ICU/TB patients	+++	[12,93]
Linezolid	6–21	>21	31	recommended for ICU/TB patients	nr	[12,93]
Meropenem	21–42	>145	~2	recommended for ICU/TB patients	+++	[12,93]
Moxifloxacin	3–12	unclear	50	recommended for ICU/TB patients	+++	[12,93]
Ofloxacin	2–28	unclear	32	recommended for ICU patients	+	[12]
Oxacillin	1–5	>411	94.2 +/− 2.1	recommended for ICU patients	nr	[90]
Piperacillin	155–310 (Css)	>1000	51.7	recommended for ICU patients	nr	[90]
Rifampicin	10–29 (Peak) *	>67	86.1–88.9	recommended for ICU patients	nr	[12]
Sulfamethoxazole	unclear	395–592	70	neither recommended nor discouraged	+++	[12]
Ticarcillin	~843		45	recommended for ICU patients	nr	[12]
Tigecycline	0.1–1		71–89	recommended for ICU patients	nr	[12]
Tobramycin	<4 (trough); 10–21 (peak)	>4 (through); >26 (peak);	<10	required	+++	[12]
Vancomycin	3–7 (trough); 14–28 (peak)	>7 (trough); >55 (peak)	55	required	nr	[12]
Voriconazole	1–6 (trough)	14–17	58	required	+++	[12]

* Two hours post-dose; ICU, intensive care unit; Css, concentration at steady state; +++, quantifiable within the range of effective concentrations (biofluid); ++, semi-quantifiable (water/other matrix); +, qualitative report; nr, not reported.

5. Conclusions and Perspectives

Accessible point of care tests and rapid technology-based assays for therapeutic drug monitoring (TDM) are urgently needed for more effective therapeutic regimens and a more objective approach to monitoring adequacy of antimicrobial treatment.

Huge advances in SERS spectroscopy have been recently taking place, leading to the possible full application of this technology. SERS methods offer short analysis times, low costs, and a variety of modifications and customizations, which can make them optimal tools for the transition of healthcare management in intensive care units towards precision medicine. Portable or hand-held SERS systems, possibly integrated in a device including an automated sample pre-treatment, would be required for on-site real-time and continuous point-of-care analysis. Such integrated systems are likely to become a powerful next-generation biomedical diagnostic tool—not requiring large analytical infrastructures, they will give the opportunity of a fast and reliable TDM, also in countries with inadequate arrangements, and the possibility of immediate results will make SERS devices especially beneficial for the analysis of instable agents, such as meropenem.

However, the majority of the methods reported in this review have been performed using high-end, non-portable microscope-based instruments. Even if it can be speculated

that the microscope was used only as a light delivery and collection vehicle, being easily replaceable with fiber-based and more portable solutions, still only few studies used portable or hand-held devices.

To apply SERS as a routine tool for TDM, future research should address some unmet needs, namely quantitative SERS methods of different antimicrobial agents, working in real biofluids at proper concentration levels, focusing on the standardization of experimental protocols, the development of novel SERS substrates with integrated internal standards, and possibly the integration with continuous sampling devices to measure the concentration of the free antimicrobial fraction in the bloodstream. The interference of biofluid components still needs further research to obtain rapid, non-destructive, and accurate detection of antimicrobials and their metabolites.

Moreover, large-scale clinical validation studies for all the proposed methods are still missing and must be addressed before routine SERS methods can be better positioned in clinical laboratories.

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