J Antimicrob Chemother 2021; **76**: 430–433
doi:10.1093/jac/dkaa445 Advance Access publication 23 October 2020

Journal of Antimicrobial Chemotherapy

First international quality control programme for laboratories measuring antimicrobial drugs to support dose individualization in critically ill patients

E. Wallenburg [1]*, R. J. Brüggemann[1,2], K. Asouit[1], M. Teulen[1], A. F. J. de Haan[3], E. J. F. Franssen[2,4] and R. E. Aarnoutse[1,2]

[1]*Department of Pharmacy, Radboud Institute of Health Sciences, Radboud Center for Infectious Diseases, Radboud university medical center, Nijmegen, The Netherlands;* [2]*Association for Quality Assessment in TDM and Clinical Toxicology (KKGT, http://kkgt.nl/), Dutch Foundation for Quality Assessment in Medical Laboratories (SKML, https://www.skml.nl/), The Hague, The Netherlands;* [3]*Department for Health Evidence, Biostatistics, Radboud university medical center, Nijmegen, The Netherlands;* [4]*Department of Pharmacy, Onze Lieve Vrouwe Gasthuis, Amsterdam, The Netherlands*

*Corresponding author. E-mail: Eveline.Wallenburg@radboudumc.nl

Received 20 July 2020; accepted 28 September 2020

Objectives: International quality control (proficiency testing) programmes are instituted to safeguard the analytical performance of laboratories and to aid these laboratories in identifying sources of error in their analytical methods. We describe the first international quality control programme for antimicrobial agents that are frequently used in critically ill patients.

Methods: Spiked plasma samples with ceftazidime, ciprofloxacin, flucloxacillin, piperacillin, sulfamethoxazole, *N*-acetyl sulfamethoxazole and trimethoprim were shipped to 22 laboratories from eight different countries. Acceptable accuracy by the performing laboratory was defined if measurements were within 80%–120% limits of the true weighed-in concentrations.

Results: A total of 81% of the measurements (ranging between 56% and 100%, dependent on drug) were within the 80%–120% limits of the true weighed-in concentrations.

Conclusions: We found a relatively good performance of the participating laboratories in measuring eight different antimicrobial drugs. Nevertheless, some of the antimicrobial drugs were not measured properly as up to 44% of the measurements was inaccurate depending on the drug. Our results emphasize the need for and utility of an ongoing quality control programme.

Introduction

Inadequate antimicrobial dosing has been shown to raise the risk of clinical failure and predisposes to the development of antimicrobial resistance as well as toxicity.[1] These unfavourable events can probably be ascribed to suboptimal concentrations achieved in plasma and at the site of action. Achieving adequate antibiotic exposure is especially challenging in critically ill patients, due to the enormous inter-patient pharmacokinetic variability in this population.[2] In order to optimize dosing regimens of available antibiotics, numerous studies have been performed investigating their pharmacokinetics in this and other patient populations.[3–7] Although there is still debate over the concentration–effect and concentration–toxicity relationships of many of these antimicrobial drugs, dose individualization guided by measurement of plasma drug concentrations [therapeutic drug monitoring (TDM)] is gaining popularity as a means to improve the use of currently available antimicrobial drugs. In addition, TDM of antimicrobial drugs is recommended for critically ill patients with acute kidney injury and for patients receiving renal replacement therapy by the Kidney Disease Improving Global Outcomes (KDIGO) organization.[8]

Both for research purposes as well as for the clinical use of TDM, several laboratories developed assays to determine antimicrobial drug concentrations. These methods are internally validated to ensure they have sufficient accuracy, precision and specificity. Participation in an external quality control (QC) or proficiency testing programme is an essential component of quality assurance and it proves the quality of the analytical method for external

parties. Moreover, accrediting bodies demand laboratories to participate in an external QC programme.[9] Whereas QC programmes for aminoglycosides and glycopeptides have been in place for several years, there has been no independent, international programme for other antimicrobial drugs so far. Therefore, we developed an international QC programme for the measurement of antimicrobial drugs that are commonly used and warrant dose optimization, especially in critically ill patients.

Methods

Antimicrobial drugs involved in the first two rounds of this programme were ceftazidime, ciprofloxacin, flucloxacillin, piperacillin, tazobactam (a β-lactamase inhibitor combined with piperacillin), sulfamethoxazole and its metabolite *N*-acetyl sulfamethoxazole, and trimethoprim. These drugs were selected since they are frequently prescribed for treating infections in critically ill patients. All obtained drug substances were of analytical quality with a high specified purity (>99%) as specified in a certificate of analysis. Drug-free EDTA plasma from selected healthy volunteers was obtained from the Dutch Blood Bank (Sanquin, Nijmegen, The Netherlands). Two QC samples (one sample per round) were prepared by spiking 1.0 mL of drug-free plasma with all antimicrobial drugs at either low or high concentrations, all within the clinical exposure range.[10,11] The antimicrobial drugs were weighed out on independently calibrated balances, dissolved in DMSO or 0.1 M HCl, and diluted using calibrated pipettes and volumetric flasks. The QC samples were dispensed in polypropylene vials and stored at −40°C until shipment. Samples were dispatched on dry ice in view of the instability of some of the drugs. Stability of drugs at −40°C had been assessed before.

The samples were analysed with our own validated ultra HPLC-MS/MS method as a confirmative check. They were released for the QC programme if the deviation was less than 10% from the weighed-in concentrations.

All weighed-in concentrations were considered true values. Acceptable accuracy by the performing laboratory was defined if measurements were within 80%–120% limits of the true weighed-in concentrations. The 20% limits were based on guidelines for method validation for bioanalysis of drugs where a deviation of 20% deviation is used as fixed criterion for inaccuracy at the lowest level of quantification.[12,13]

To estimate the influence of the antimicrobial drug to be measured, the concentration level (high or low) and the analytical method, a multilevel model analysis was performed on the absolute inaccuracies. A random intercept model was used to deal with intra-laboratory correlation between absolute inaccuracies achieved for the various drugs. The Tukey method was used to correct for multiple comparisons. All statistical analyses were performed using R (version 3.6.2) with R Studio (version 1.1.463), with packages 'nlme', 'lmerTest' and 'emmeans'.[14–16]

Both rounds were accompanied by clinical cases related to the TDM results that required interpretation by means of a multiple-choice question. The first case related to subtherapeutic exposure of flucloxacillin. The second case related to ciprofloxacin in a critically ill patient. All participants were provided feedback anonymously on their performance as well as feedback on the clinical cases.

Results

In the first round 17 laboratories and in the second round 22 laboratories from eight different countries participated in the QC programme. Only one laboratory was able to measure all eight compounds. The other laboratories measured a selection of one up to seven of the antimicrobial drugs. The laboratories used conventional HPLC or ultra HPLC with fluorescence, UV or diode-array detection or LC with MS detection to measure total (i.e. protein-unbound plus bound) antimicrobial drug concentrations. A total of 136 analyses were performed in both rounds. A quantitative result was obtained for 131/136 (96%) of the measurements. There were five reported measurements below the limits of quantification of assays. For one laboratory the measurements of flucloxacillin, piperacillin and tazobactam were excluded from the results, since only unbound concentrations of these three drugs were reported.

A total of 81% (range 56%–100%, dependent on drug) of the measurements were determined accurately (Table 1 and Figure 1). For the multilevel model analysis we excluded four measurements with a gross deviation from the true value (with absolute inaccuracies ranging from 191% to 6150%).

There was no significant intra-laboratory correlation found between the absolute inaccuracies (in percentage deviation from the true values) [$\chi^2(1) = 1.73$, $P = 0.19$]. The mean absolute inaccuracies for measurement of the different antimicrobial drugs were significantly different [$F(7, 98) = 3.07$, $P < 0.01$]. The measurements of flucloxacillin and *N*-acetyl sulfamethoxazole showed the best performance; 100% (21 out of 21 and 13 out of 13, respectively) of the samples were determined accurately. The measurements of ceftazidime showed the worst performance; 56% (14 out of 25) of the samples were determined accurately. The absolute inaccuracy achieved for ceftazidime was significantly higher than for flucloxacillin (95% CI = 2.73–17.96, $P = 0.0014$), sulfamethoxazole (95% CI = 3.77–19.76, $P = 0.004$) and *N*-acetyl sulfamethoxazole (95% CI = 2.49–20.31, $P = 0.0033$). There was no significant effect of the concentration level to be analysed or of the used analytical method on the absolute inaccuracy [$F(1, 98) = 0.32$, $P = 0.58$ and $F(1, 98) = 0.028$, $P = 0.87$, respectively]. The statistical interaction between antimicrobial drug, concentration level and analytical method was not significant.

Ten laboratories responded to the clinical case about flucloxacillin and six laboratories responded to the clinical case about ciprofloxacin. A total of 50% of the respondents filled in the correct answer, as defined by the authors.

Discussion

The initial results of this programme showed a relatively good performance of the participating laboratories in measuring antimicrobial drugs that are commonly used in critically ill patients. A total of 81% of the measurements (ranging between 56% and 100%, dependent on drug) were within the 80%–120% limits of the true weighed-in concentrations. First rounds of previous programmes initiated by us presented a similar performance: 65%, 77% and 83% with an acceptable accuracy for antiretroviral drugs, azole antifungal drugs and TB drugs, respectively.[17–19] Compared with a national Belgium proficiency programme including meropenem and piperacillin (with acceptable accuracies of 56% and 72%, respectively), the performance of the first round of this international programme was better.[20]

Nevertheless, still one out of five (19%) measurements was inaccurate. Inaccurate results may introduce bias in pharmacokinetic studies or may lead to inappropriate dose adjustments in TDM. Our data suggest that in particular the measurement of ceftazidime and trimethoprim requires further improvement. It is

Table 1. Measurements of QC samples, subdivided by antimicrobial drug and concentration level

Drug	Number of measurements	Concentration level	Measured concentration relative to true value (%)	Absolute inaccuracy (%)[a]	Measurements with acceptable accuracy[b]		
					n	%	overall %
Ceftazidime	14	low	89 (69–160)	16 (4–60)	9	64	56
	11	high	85 (75–125)	20 (2–25)	5	45	
Ciprofloxacin	4	low	98 (74–98)	2 (2–26)	3	75	82
	7	high	88 (78–106)	12 (6–22)	6	86	
Flucloxacillin	10	low	96 (89–112)	7 (1–12)	10	100	100
	11	high	95 (80–101)	5 (1–20)	11	100	
Piperacillin	8	low	105 (69–6250)	11 (1–6150)	5	63	75
	12	high	106 (89–121)	10 (2–21)	10	83	
Tazobactam	3	low	107 (107–115)	7 (7–15)	3	100	88
	5	high	99 (82–125)	7 (0–25)	4	80	
Sulfamethoxazole	10	low	96 (80–112)	7 (0–20)	9	90	94
	8	high	97 (91–100)	3 (0–9)	8	100	
N-acetyl sulfamethoxazole	7	low	95 (86–114)	7 (0–14)	7	100	100
	6	high	96 (91–100)	4 (0–9)	6	100	
Trimethoprim	7	low	97 (73–1453)	27 (3–1353)	2	29	62
	6	high	91 (87–92)	9 (8–13)	6	100	

Data are presented as median (range) unless otherwise stated.
[a]Inaccuracy is the percentage bias from the true concentration., i.e. inaccuracy = (100 × measured concentration/true concentration) − 100%.
[b]Acceptable measurements are within the 80%–120% limits of the true concentrations.

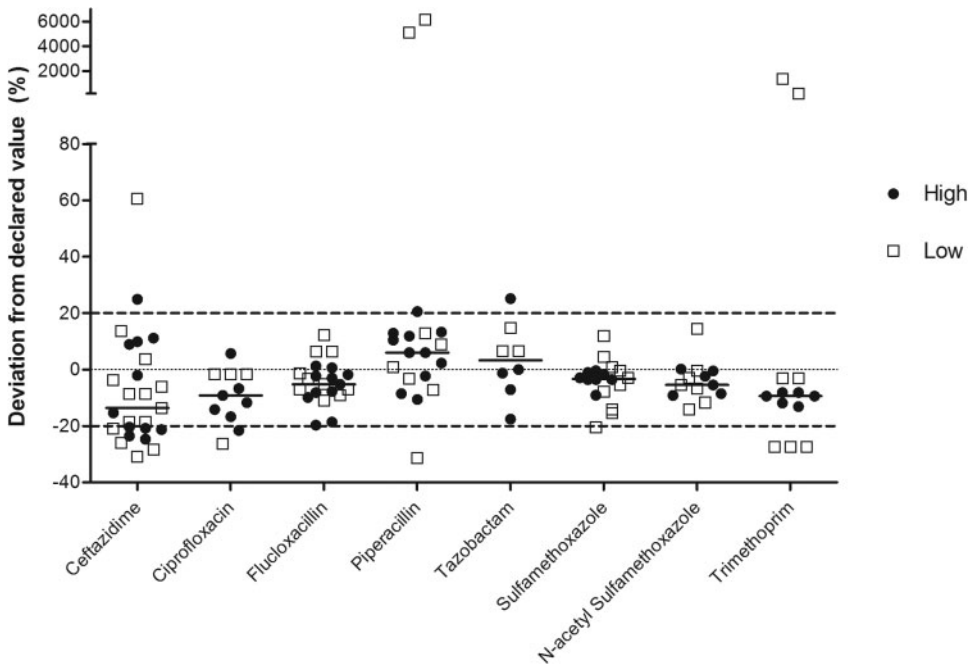

Figure 1. Deviation from the declared 'true' value. Each point represents a single measurement, shown as the percentage of the true weighed-in concentration. The horizontal solid lines represent the median values. Accuracy was acceptable if measurements fell within 80%–120% limits of the weighed-in concentrations (dashed lines). Filled circle, high spiked concentration; open square, low spiked concentration.

unclear what factors might have caused the observed deviations, since the sources of error were not assessed. There are many possible sources of bias that may explain the results, including methodological, technical and clerical errors.[21,22] Also the instability of some of the antimicrobial drugs (i.e. flucloxacillin, ceftazidime, piperacillin and tazobactam) might have contributed to the

observed variability. Sending an error evaluation form to the participating laboratories will be considered for future rounds.

The exclusions of the four outliers in the multilevel analysis was a subjective decision by the authors. Although we did not assess the sources of bias, we assumed that a deviation of more than three times the true value is caused by clerical errors instead of analytical or methodological errors. The mean absolute inaccuracy is greatly affected by these outliers. If the four measurements were not excluded there was still a significant effect of the type of antimicrobial drug to be measured on the absolute inaccuracy [$F(7, 112) = 2.39$, $P = 0.03$] and a non-significant effect of the concentration level [$F(1, 112) = 2.74$, $P = 0.10$]. However, a significant interaction effect was present [$F(7, 112) = 2.53$, $P = 0.02$], which indicates that the mean absolute inaccuracy of the type of antimicrobial drug to be measured was affected differently by concentration level. Absolute inaccuracy was significantly lower for measurement of piperacillin with a low concentration level than for other antimicrobial drugs and concentration levels ($P < 0.05$).

The test case histories are meant for educational purposes and are focused on key clinical issues in TDM of antimicrobial drugs. There is ongoing debate on which clinical TDM target concentrations should be used for the antimicrobial drugs involved in this QC programme, especially for β-lactam antibiotics.[23] The variation of TDM targets and dosing adjustment strategies used in the clinical setting is an interesting topic for discussion in the clinical cases.

By participating in the programme the laboratories were alerted to possible analytical problems, which may help them to improve their methods. Our results emphasize the need for and utility of an ongoing QC programme. We acknowledge the selection of antimicrobial drugs used in this programme is not complete. Therefore, in future rounds the programme will be extended to the measurement of other important antimicrobial drugs like meropenem as well as the possibility to report free (unbound) concentrations. Laboratories are encouraged to participate in this QC programme.

Funding

This study was supported by the Radboudumc Department of Pharmacy.

Transparency declarations

None to declare.

References

1 Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. *P T* 2015; **40**: 277–83.

2 Roberts JA, Abdul-Aziz MH, Lipman J *et al*. Individualised antibiotic dosing for patients who are critically ill: challenges and potential solutions. *Lancet Infect Dis* 2014; **14**: 498–509.

3 Alobaid AS, Wallis SC, Jarrett P *et al*. Population pharmacokinetics of piperacillin in nonobese, obese, and morbidly obese critically ill patients. *Antimicrob Agents Chemother* 2017; **61**: e01276-16.

4 König C, Braune S, Roberts JA *et al*. Population pharmacokinetics and dosing simulations of ceftazidime in critically ill patients receiving sustained low-efficiency dialysis. *J Antimicrob Chemother* 2017; **72**: 1433–40.

5 Autmizguine J, Melloni C, Hornik CP *et al*. Population pharmacokinetics of trimethoprim-sulfamethoxazole in infants and children. *Antimicrob Agents Chemother* 2018; **62**: e01813-17.

6 Wilkes S, van Berlo I, Ten Oever J *et al*. Population pharmacokinetic modelling of total and unbound flucloxacillin in non-critically ill patients to devise a rational continuous dosing regimen. *Int J Antimicrob Agents* 2019; **53**: 310–7.

7 Gieling EM, Wallenburg E, Frenzel T *et al*. Higher dosage of ciprofloxacin necessary in critically ill patients: a new dosing algorithm based on renal function and pathogen susceptibility. *Clin Pharmacol Ther* 2020; **108**: 770–4.

8 Matzke GR, Aronoff GR, Atkinson AJ Jr *et al*. Drug dosing consideration in patients with acute and chronic kidney disease-a clinical update from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* 2011; **80**: 1122–37.

9 ISO 15189: Medical Laboratories—Requirements for Quality and Competence. 2012.

10 Abdul-Aziz MH, Alffenaar JC, Bassetti M *et al*. Antimicrobial therapeutic drug monitoring in critically ill adult patients: a Position Paper. *Intensive Care Med* 2020; **46**: 1127–53.

11 Brown GR. Cotrimoxazole—optimal dosing in the critically ill. *Ann Intensive Care* 2014; **4**: 13.

12 Bioanalytical Method Validation Guidance for Industry. 2018. https://www.fda.gov/media/70858/download.

13 Guideline on Bioanalytical Method Validation. 2011. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf.

14 nlme: Linear and Nonlinear Mixed Effects Models. R Package Version 3.1-143. 2019. https://CRAN.R-project.org/package=nlme.

15 Kuznetsova A, Brockhoff PB, Christensen RHB. lmerTest package: tests in linear mixed effects models. *J Stat Soft* 2017; **82**: 1–26.

16 emmeans: Estimated Marginal Means, aka Least-Squares Means. R Package Version 1.4.7. 2020. https://CRAN.R-project.org/package=emmeans.

17 Burger D, Teulen M, Eerland J *et al*. The International Interlaboratory Quality Control Program for Measurement of Antiretroviral Drugs in Plasma: a global proficiency testing program. *Ther Drug Monit* 2011; **33**: 239–43.

18 Bruggemann RJ, Touw DJ, Aarnoutse RE *et al*. International interlaboratory proficiency testing program for measurement of azole antifungal plasma concentrations. *Antimicrob Agents Chemother* 2009; **53**: 303–5.

19 Aarnoutse RE, Sturkenboom MG, Robijns K *et al*. An interlaboratory quality control programme for the measurement of tuberculosis drugs. *Eur Respir J* 2015; **46**: 268–71.

20 Carlier M, Athanasopoulos A, Borrey D *et al*. Proficiency testing for meropenem and piperacillin therapeutic drug monitoring: preliminary results from the Belgian Society on Infectiology and Clinical Microbiology Pharmacokinetic-Pharmacodynamic Working Group. *Ther Drug Monit* 2018; **40**: 156–8.

21 Droste JA, Aarnoutse RE, Koopmans PP *et al*. Evaluation of antiretroviral drug measurements by an interlaboratory quality control program. *J Acquir Immune Defic Syndr* 2003; **32**: 287–91.

22 Lempers VJ, Alffenaar JW, Touw DJ *et al*. Five year results of an international proficiency testing programme for measurement of antifungal drug concentrations. *J Antimicrob Chemother* 2014; **69**: 2988–94.

23 Wong G, Brinkman A, Benefield RJ *et al*. An international, multicentre survey of β-lactam antibiotic therapeutic drug monitoring practice in intensive care units. *J Antimicrob Chemother* 2014; **69**: 1416–23.