Clinical Biochemistry 66 (2019) 91–94

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Insufficient harmonization of antibiotics assays – Polish experience with an external quality assessment program in the years 2011–2018

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ARTICLE INFO

Keywords:
External quality assessment
Gentamycin
Harmonization
Therapeutic drug monitoring
Vancomycin

ABSTRACT

Introduction: Treatment with vancomycin and gentamycin requires strict monitoring of its serum concentration for proper dosage optimization. This study aimed to assess the quality and the harmonization of antibiotics assays in Polish laboratories.

Materials and methods: 413 results of vancomycin and 148 results of gentamycin assays obtained from Polish laboratories in 30 international external quality assessment (EQA) surveys carried out from March 2011 to May 2018 were analyzed.

Results: Interlaboratory robust coefficients of variation (rCVs) in particular surveys comprised between 1.3 and 47.2% for vancomycin, and between 1.8 and 34.2% for gentamycin. The percentage of the results with the difference above acceptable limit ± 10% from the target value established for own method group was 25.7% for vancomycin and 25.6% for gentamycin. When the difference was established according to target value for all methods, the percentage of results outside the acceptable limit was 2-fold higher on average (54.8% for vancomycin and 43.2% for gentamycin). The comparison of target values for methods revealed statistically significant differences between analytical systems used ($p < .0001$). The highest difference was 40% for vancomycin and 12% for gentamycin.

Conclusions: The present analysis revealed high dispersion of the antibiotics assays results in Polish laboratories. Moreover, vancomycin and gentamycin results differed significantly in a way dependent on the analytical system used. There appear to be an urgent need for harmonization of methods used for vancomycin and gentamycin measurement.

1. Introduction

Vancomycin and gentamycin remains a mainstay in the management of serious infections with Gram-positive and Gram-negative bacteria. As with other drugs with narrow therapeutic window, these one's use in patients' treatment requires strict monitoring of serum concentration for proper dosage optimization. It is of great importance especially when they are applied in children, elderly or renal insufficiency patients due to high nephrotoxicity of these antibiotics and mainly their renal route of elimination [1–3].

Strict drug concentration monitoring in serum requires the use of reliable, accurate and precise laboratory methods, which ensures the high quality of the obtained results. Moreover, it requires that results obtained by different measurements procedures are equivalent, especially since clinical decisions are based on universal cut-off points [4]. Harmonization of the results is a fundamental aspect of quality in laboratory medicine, which major issue is to improve patient's outcome through providing high quality results [5].

Medical laboratories ensure quality through standardization of operating procedures, internal quality control procedures, and participation in an external quality assessment (EQA) programs [6,7]. The EQA programs enable to evaluate the performance of participating laboratories and to evaluate the harmonization of methods they used.

The present study aimed to assess the quality and the harmonization of vancomycin and gentamycin assays in Polish laboratories based on retrospective analysis of the results obtained in an international EQA program.

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https://doi.org/10.1016/j.clinbiochem.2019.02.001
Received 25 July 2018; Received in revised form 24 January 2019; Accepted 3 February 2019
Available online 04 February 2019

2. Material and methods

2.1. Data collection

413 results of vancomycin and 148 results of gentamycin measurements obtained by Polish laboratories in an international EQA program provided by Labquality (Finland) from March 2011 to May 2018 were analyzed. The number of laboratories participating in program was 28 for vancomycin and 8 for gentamycin.

2.2. EQA program organization

Laboratories, which have participated in the Labquality EQA program Clinical Chemistry 11: Special Chemistry: Therapeutic Drug, obtained 2 liquid serum samples spiked with drugs at low, medium or high level, up to 4 times a year. For the analyzed surveys the mean drug concentration was as follows: for vancomycin 5.96 ± 1.15, 19.56 ± 1.94 and 35.6 ± 6.21 mg/l, and for gentamycin 1.43 ± 0.18, 3.87 ± 0.20, and 6.96 ± 0.88 mg/l. In each sample, drug concentration should have been measured in a single run. Results and information on the analytical system used for the analysis were sent to the EQA program provider and were evaluated according to the drug level, the analytical system used (method group), and the country of the participant (district group). Laboratory's result was evaluated as acceptable when the difference between the result and the target value (established as mean for own method group) did not exceed ± 10%.

2.3. Statistics

Dispersion of the Polish laboratories' results was assessed by the evaluation of robust coefficient of variation (rCV) for each sample in every survey. rCVs were calculated using equation: rCV % = 0.74 × (P75 − P25)/Median × 100, where P25 and P75 are the first and third quartiles of the obtained results [8]. The surveys in which < 3 Polish laboratories have participated were excluded from the analysis.

Accuracy of the laboratory's result was assessed by the calculation of the percentage (%) difference between the result and the established target values: 1) the mean value calculated for own method group (target $value_{own\ method}$), and 2) the mean value calculated from means for all methods used in the survey (target $value_{all\ methods}$). The target $value_{own\ method}$ was calculated from the results in particular method group obtained from all participants (from all countries). The method groups, which comprised < 3 participants per survey, were excluded from the analysis, and the target $value_{own\ method}$ was calculated from 3 to 43 results for vancomycin and from 3 to 24 results per survey for gentamycin (median 5 and 6, respectively).

For each analytical system (method group) the percentage (%) difference between the target $value_{own\ method}$ and target $value_{all\ methods}$ was calculated in each survey. As some method groups in particular surveys were excluded from the analysis due to number of participants < 3, and in some surveys some method groups were absent, for particular method groups the difference between the target $value_{own\ method}$ and target $value_{all\ methods}$ was calculated from the range of 11 to 60 surveys (median 45) for vancomycin and from 6 to 60 surveys (median 38) for gentamycin, respectively. The normality of the data for each method group was assessed using Shapiro-Wilk test. As the data distribution wasn't normal the results are expressed as median and 25th and 75th percentiles, minimum and maximum. Kruskal-Wallis test was used to compare groups. P < .05 was considered statistically significant.

Table 1
Interlaboratory robust coefficients of variation (rCVs) for Polish laboratories' results in EQA surveys for antibiotics in 2011–2018. Parameters were calculated for surveys in which participated at least 3 Polish laboratories.

	Vancomycin		
Drug level	Low (n = 12)	Medium (n = 21)	High (n = 19)
Median rCV [%]	18.2	9.6	18.8
Percentiles 25th–75th	11.3–24.8	5.4–12.9	10.1–20.7
Min–Max	4.9–47.2	2.9–18.2	1.3–31.3

	Gentamycin		
Drug level	Low (n = 10)	Medium (n = 13)	High (n = 6)
Median rCV [%]	9.4	6.3	9.5
Percentiles 25th–75th	5.4–13.5	4.5–8.2	4.7–13.4
Min–Max	2.8–34.2	1.8–19.8	3.1–23.4

3. Results

3.1. Harmonization of antibiotics assays in Polish laboratories

The rCVs of Polish laboratories' results in particular surveys ranged from 1.3% to 47.2% and from 1.8% to 34.2%, for vancomycin and gentamycin, respectively (Table 1). In 19% of surveys for vancomycin and 10% of surveys for gentamycin the rCVs exceeded 20%.

25% of vancomycin and gentamycin results were beyond acceptable range: target $value_{own\ method}$ ± 10%. When laboratories' results were compared to target $value_{all\ methods}$ 54.7% of vancomycin and 43.2% of gentamycin measurements were beyond acceptable limit. For both antibiotics percentage of results biased > 20% from target $value_{all\ methods}$ was about 20% (Table 2).

3.2. Effect of the analytical system on the harmonization of the results

The difference between target $value_{own\ method}$ and target $value_{all\ methods}$ ranged in particular surveys from −35% to 70% for vancomycin and from −20% to +21% for gentamycin (Fig. 1). For both analyzed antibiotics, there were statistically significant differences between the results obtained with different analytical systems (p < .0001). The greatest difference between two vancomycin determination methods was 40%. For 4 method groups the median difference between target $value_{own\ method}$ and target $value_{all\ methods}$ exceeded acceptable limit ± 10%. For gentamycin the difference between methods was lower and reached a maximum of 12% (Fig. 1).

Table 2
Number and percentage of Polish laboratories results within bias range established according to the target $value_{all\ methods}$ and target $value_{own\ method}$ (in parenthesis).

	Vancomycin					
Bias range	≤10%		10–20%		≥20%	
Number of results	187	(269)	127	(70)	99	(23)
% of results	45.3	(74.3)	30.8	(19.3)	24.0	(6.4)

	Gentamycin					
Bias range	≤10%		10–20%		≥20%	
Number of results	84	(32)	37	(7)	27	(4)
% of results	56.8	(74.4)	25.0	(16.3)	18.2	(9.3)

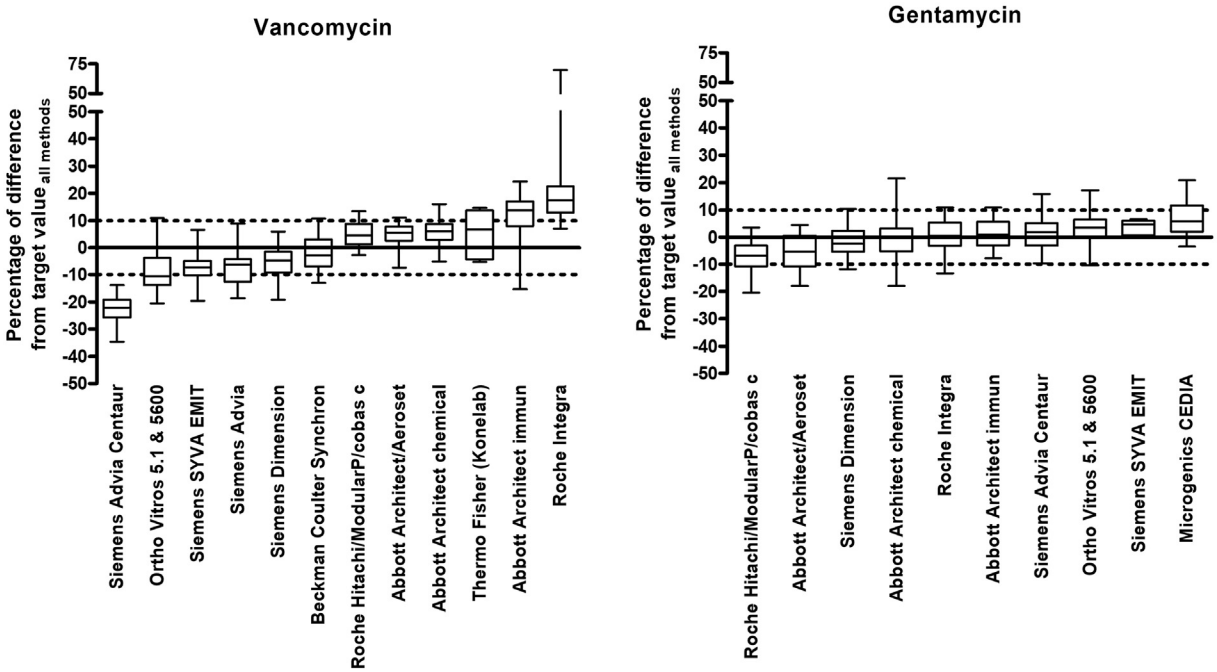

Fig. 1. Percentage difference between target value$_{\text{all methods}}$ and target values$_{\text{own method}}$, for analytical systems used by the participants of EQA surveys for antibiotics in 2011–2018. Data are expressed as median, 25th–75th percentiles and minimum and maximum (box and whiskers).

4. Discussion

Therapeutic drug monitoring has become an expanding area of laboratory diagnostics and clinical pharmacology. Levels of the antibiotics in serum have to be especially constantly monitored because of their narrow therapeutic window and to prevent toxic effects, as well as for the need to exceed the minimal inhibitory concentration. Since gentamycin and vancomycin are restricted to use in inpatients (hemodialyzed, immunosuppressed after transplantation, with kidney failure or infected with resistant strains of bacteria), who are frequently in serious clinical conditions, reliability of the measurement is of great importance [1,2,6].

The current study was performed in order to assess the quality and harmonization level of antibiotics assays used in Poland, in the last 8 years. Moreover, since up to date the data on quality of antibiotics assays and harmonization of currently used in laboratories the analytical systems are very scarce [9,10], the presented analysis seems to be of great importance. Therapeutic drug monitoring is not (in Poland) provided on a regular basis in every laboratory. This part of laboratory service is rather centralized and fulfilled only by the biggest laboratories or the one having specific group of patients. The nationwide EQA program for therapeutic drug monitoring in Poland exists, but comprises only phenytoin, carbamazepine, valproic acid and digoxin [11]. Thus Polish laboratories participate also in other EQA programs, which allow the quality assessment of antimicrobial agents' analysis. Such EQA programs available in Poland are few, so the number of laboratories participating in each may be quite low. Nevertheless, in the last 8 years we have observed the increased interest in EQA program for vancomycin (from 7 laboratories in 2011 to 18 laboratories in 2017) and gentamycin (from 2 to 7), which may be result of increased need for these antibiotics use in general.

The present analysis revealed problems in overall quality of antibiotics assays in Polish laboratories, despite their increasing participation in EQA program. There were a high percentage of results beyond acceptable range, especially when compared to target value$_{\text{all methods}}$. Problems with quality of the results cannot be addressed only to low level of drugs since for every drug level there was a wide range of the obtained results and high percentage of results differed significantly from the target value. It was not also caused by an extremely skewed EQA results obtained in individual laboratories, since for none of participating laboratories the median of percentage difference from target value$_{\text{own method}}$ exceeded 10% (data not shown).

Thus the results of our analysis led to the conclusion, that one of the important reasons of the poor harmonization of the Polish laboratories' results might be differences between analytical systems used. Indeed, in our work we provide evidence, that results obtained with few particular analytical systems significantly differ and this difference may exceed 40%.

The significant differences between methods available for assay of vancomycin and aminoglycosides has been observed by others [9,12,13]. For instance, Wilson et al. in the study based on EQA program results performed in United Kingdom has found that for gentamycin CEDIA, Bayer chemiluminescence and Abbott TDx assays produced significantly lower results, whereas Roche FPIA and Dade Behring Emit produced higher results, in comparison to other methods. For vancomycin, Abbott AxSYM produced the lowest results whereas Sigma FPIA the highest. In our study, the number of method groups was about 2-fold higher, in comparison to the study published in 2003 [9]. The differences between analyzers observed by us were higher for vancomycin than for gentamycin. The highest difference between two analytical systems (Siemens Advia Centaur and Roche Integra) reached 40%.

Most commonly in routine diagnostics the measurement of drug concentration is performed using commercially available immunochemical technologies delivered and ready to use. The diversity of analytical systems available for drugs monitoring and continuing development of the new ones can lead to frequent changes of methods in laboratories. Samardzic et al. compared results of vancomycin through concentration monitored in neonates, using different immunoassays and found out that after switching the method from PETINIA to Cobas, results obtained through the two year observation were 20% lower. Authors suggest switching to more advanced analytical techniques, for example LC/MS [12]. In other paper, comparison LC-MS/MS with immunoassays revealed substantial differences between methods, which lead to discordant interpretation of 1 out of 5 results [13].

Our study has some limitations. It should be taken into account, that

quality of the EQA results may be affected by sample preparation or handling, and EQA samples could not be commutable with patients' samples [14]. Spiked EQA samples simulate patient's material, but may differ in subtle ways. Important difference in EQA and clinical material is the presence of preservatives and many different drugs molecules. pH of the prepared control sample is higher than native serum what may significantly affect susceptible methods, i.e. based on enzyme activity measurement. On the other hand EQA material does not contain drug metabolites, so the risk of cross-reaction in immunoassays seems to be lower. Thus the laboratories' results in EQA program are mainly evaluated according to own analytical system, having regard that the control sample may behave differently in different analyzers.

Unfortunately, the data about the commutability of the EQA specimens for therapeutic drug monitoring are very scarce. Recently, the paper assessed the commutability for the material used for antibiotics analysis based on example of tobramycin has been published [14]. The results of this study indicate that specimens made with human serum behaves more like patient samples compared to bovine serum. The control sample used in Labquality EQA program for drug monitoring is of human origin, but we did not have the detailed data about its commutability. However, as other previous studies based on both, EQA material and patients' samples also showed the differences between methods in antibiotics analysis [9,13,15,16] we can conclude that the differences between methods observed by us are not the result of poor commutability of the analyzed samples, but rather point to the differences between methods. Our analysis of the harmonization of results without reference to the analytical method we have considered reasonable, since the recipient of the analysis result (clinician) usually does not achieve the information on the method used.

Discrepancy between results obtained with different method might lead to inappropriate conclusions by those, who are responsible for drug dosage optimization and needs to be pointed out. Pitfalls in antibiotic monitoring may lead to too low doses administration and give the risk of subtherapeutic drug concentration and eventually, lead to development of antimicrobial resistance [17].

Thus, laboratories should consider evaluation of own vancomycin and gentamycin therapeutic ranges or the harmonization of methods for antibiotics assay is needed. Harmonization of the laboratory results means that clinical laboratory test results will be equivalent independently from the clinical laboratory that produced the results. Recently, the International Consortium for Harmonization of Clinical Laboratory Results has been established, which mission is to provide a centralized process to organize global efforts to achieve harmonization of clinical laboratory results [18]. The Council prepared a document with measurands and herein status of harmonization. Vancomycin is present on this list with status ‘needed’, what is with agreement with conclusions from our study. Gentamycin is not presented in this document. Our study showed that the gentamycin assays were better harmonized than vancomycin assays; however, there was also a high dispersion of laboratories results and a statistically significant difference between methods. Thus gentamycin assays could also be considered as requiring harmonization.

5. Conclusions

The present analysis revealed high dispersion of the antibiotics assays results in Polish laboratories. Moreover, vancomycin and gentamycin results differed significantly in a way dependent on the analytical system used. Clinical chemists, pharmacologists, and pharmacists should pay special attention to possible differences between analytical systems used for drug monitoring. There appeared to be an urgent need for harmonization of vancomycin and gentamycin methods used for antibiotics monitoring.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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