Rapid detection of vancomycin-resistant enterococci by the laser monitoring of microbial growth

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Introduction
Vancomycin-resistant enterococci (VRE) represent a major nosocomial pathogen with increasing prevalence. Rapid detection of VRE is essential for appropriate treatment and infection control purposes. While rapid identification of enterococci has become possible with matrix-assisted laser desorption/ionization/time-of-flight mass spectrometry (MALDI-TOF MS), the current methods of antimicrobial susceptibility testing still necessitate long incubation times. Thus, a rapid method is needed for differentiation between vancomycin-susceptible enterococci (VSE) and VRE.

Methods
Eleven vancomycin-resistant E. faecium isolates and ten vancomycin-susceptible E. faecium isolates from clinical samples were used. Species identification was performed by MALDI-TOF MS. Minimum inhibitory concentrations (MIC) of vancomycin were determined by broth microdilution reference method according to the ISO 20776-1 guideline. Vancomycin susceptibility was confirmed by the PCR for vanA and vanB genes. Rapid susceptibility testing was performed using BacterioScan™216R instrument (BacterioScan Inc., St. Louis, US), based on the laser-scattering for quantification of microbial growth. The start inoculum of 5x10⁵ cfu/ml was prepared in cation-adjusted Mueller–Hinton broth. The samples were incubated in the BacterioScan™216R device at 35°C for 6 hours, adding vancomycin in the breakpoint concentration of 4 mg/L. The same was done without adding vancomycin, to have a growth control for each isolate. The measurements were automatically taken approximately every 3 min for each sample. The ratio of growth trends was estimated for each isolate as compared to its growth control. This was done consecutively for all time points using the SCARM filter, a method that can operate in real-time. Based on the growth trend ratios, receiver operating characteristic (ROC) analyses were carried out for all time points.

Results
The estimated area under the ROC curve (AUC) exceeded 0.9 already after 1.5 hours, indicating that the growth trend ratios can be used for accurate and early discrimination between VRE and VSE. Choosing the optimal cutoff (maximum Youden-index), the estimated sensitivity and specificity for VRE detection were 100% and 80% already after 2 hours of incubation, and 100% and 90% after 2.5 hours, respectively. Both MIC50 and MIC90 of VRE isolates were ≤128 mg/L according to the broth microdilution method. Among VREs, five and six isolates were vanA-positive and vanB-positive, respectively.

Conclusions
- The described real-time growth-based susceptibility testing approach is promising for rapid detection of vancomycin-resistant enterococci.
- The validation of this approach on a larger collection of clinical isolates for exact determination of its diagnostic performance is ongoing.
- Further studies should also focus on the direct testing from clinical specimens.

Acknowledgements
We are thankful to Damayanti Kaiser for excellent technical assistance. The BacterioScan™216R device and consumables were provided free of charge for the study period by BacterioScan Inc.