Rapid Growth-Based Detection of Carbapenem-Resistant Gram-Negative Bacteria Using the Real-Time Light-Scattering Method

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Introduction
The prevalence of multidrug resistant Gram-negative bacteria is increasing worldwide. Therefore, rapid detection of carbapenem resistance is critical for early initiation of an appropriate antimicrobial treatment.

Methods
Growth-based detection of meropenem resistance was performed by the light-scattering method using BacterioScan™216R device for real-time monitoring of microbial growth (BacterioScan Inc., St. Louis, MO). Ten meropenem-resistant and ten meropenem-susceptible clinical isolates of Gram-negative rods (seven Pseudomonas aeruginosa and three Klebsiella pneumoniae isolates in each group) were used. The inoculum of 5x10⁵ cfu/ml was prepared in cation-adjusted Mueller-Hinton broth. The samples were incubated in the BacterioScan instrument for 6 hours at 35°C with and without meropenem in breakpoint concentration of 2 µg/ml. Each sample was automatically measured every 3 minutes. ROC analyses were performed considering the ratio of growth trends (bacteria vs. corresponding sample with antibiotic) for all time points, where growth trends were estimated using a recently developed moving window regression procedure. Broth microdilution method was performed for determination of meropenem minimum inhibitory concentrations (MIC).

Results
The estimated sensitivity and specificity for detection of meropenem-resistant Gram-negative bacteria achieved both 100% after 200 minutes of incubation. Due to the particularly early response of susceptible K. pneumoniae isolates to meropenem, the differentiation of resistant vs. susceptible isolates was possible already after 80 minutes for this species. According to the broth microdilution, MIC⁵₀/MIC⁹₀ were 16/16 µg/ml and 0.25/0.5 µg/ml for meropenem-resistant and meropenem-susceptible isolates, respectively.

Conclusions
❖ The light-scattering method is promising for the early detection of carbapenem-resistant Gram-negative bacteria
❖ The time to result seems to be particularly short for Enterobacteriaceae
❖ The performance of this method warrants further investigation on a multitude of geno- and phenotypically diverse isolates

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