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Paper Poster Session IV

Update on molecular-based diagnosis

First multicentre external quality assessment to assess the quality of molecular amplification methods for the detection of vancomycin-resistant enterococci

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Objectives: An external quality assessment (EQA) panel consisting of a total of 14 samples in transport medium was prepared to assess the proficiency of laboratories in the correct detection of vancomycin resistant enterococci (VRE) by molecular methods.

Methods: Stock suspensions of bacterial strains were cultured and quantified in CFU/ml. The panel consisted of various vancomycin resistant enterococci at various dilutions between $1.0x10^5$ and $1.0x10^7$ CFU/ml. The panel also included samples containing various vancomycin susceptible enterococcus strains and a mixed sample containing *E. gallinarum* and *E. faecium*. In order to investigate assay specificity, an *S. aureus* positive and an Enterococcus negative sample were also included. All samples were prepared in brain heart infusion matrix.

A testing panel was analyzed prior to distribution on dry ice to participants to confirm the VRE status of each panel member. The panel samples were randomized, labeled, packed and distributed by QCMD. Participants were given four weeks to test the blinded panel. Results were reported back to QCMD via a dedicated online system. All results were analysed in order to assess the performance of laboratories in the correct detection of vanA and vanB.

Results: 44 laboratories from 16 countries participated in this EQA study. 34 datasets were returned. For the detection of the van-genes the following molecular methods were used: Hain Lifescience Geno Type Enterococcus 12 (n=2), Cepheid Xpert vanA (n=1), Cepheid Xpert vanA/vanB (n=5), Roche LightcCycler VRE detection kit (n=1), Vela Diagnostics Sentosa SA vanA/vanB PCR (n=1), in-house conventional multiplex PCR (n= 3), in-house conventional single PCR (n= 4), in-house real-time PCR (n=22). Most participants were able to correctly determine the presence of vanA (≥ 92.3%) in the true positive samples. The correct detection of vanB was lower with 71.8-87.2% of datasets determining these samples as VRE positive. A false positivity rate of 7.7% on the true negative sample was obtained. The panel contained three samples that contained Enterococcal species or other bacteria that were vancomycin sensitive. In 2/3 samples, false positivity levels were lower than for the true negative sample. One sample contained an *E. faecium* strain being phenotypically sensitive to vancomycin and teicoplanin but was found to be vanA positive by 51.3% of participants, of which 72.7% used an in-house real-time PCR. Further characterization of this strain (sample VRE13-07) did not reveal the presence of vanR/S/H/X/Y/Z, orf1/2, or IS1251 indicative for vancomycin variable enterococci.

Conclusion: The majority of returned results were generated using in-house PCRs (76.9%). The remaining results (23.1%) were obtained by using commercially available kits. Most participants were able to correctly characterize the vancomycin resistance gene as vanA or vanB. After further molecular characterization of VRE13-07, the reported positive results are not due to the presence of part of the Tn1546 transposon.