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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 was prepared to assess the proficiency of laboratories in the correct detection of vancomycin resistant enterococci (VRE) by molecular methods.

MATERIALS & 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 1.0x105 and 1.0x107 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* and an Enterococcus negative sample were also included. All samples were prepared in brain heart infusion matrix.

Prior to distribution to participants, the panel was tested to confirm the VRE status of each panel member. Panel samples were randomized, labeled, packed and distributed on dry ice 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.

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RESULTS

- 44 laboratories from 16 countries
- 34 datasets returned.
- Molecular methods used for detection of the van-genes : 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 correctly determined the presence of vanA (≥ 92.3%) in the true positive samples (Table 1).
- Correct detection of vanB was lower with 71.8-87.2% of datasets determining these samples as VRE positive (Table 1).
- False positivity rate of 7.7% on the true negative sample.
- The panel contained 3 samples with Enterococcal species or other bacteria that were vancomycin sensitive: false positivity levels were lower than for the true negative sample.
- VRE13-07: *E. faecium* strain phenotypically sensitive to vancomycin and teicoplanin but 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 did not reveal the presence of the vanA operon (Gagnon et al.) indicative for vancomycin variable enterococci (VVE) (Table 2).

References:
Gagnon et al. 2011. J. Antimicrob. Chemother. **66:** 2758-2762.
Szakacs et al. 2014. J. Clin. Microbiol. **52:**1682-1686.

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CONCLUSIONS

- Majority of returned results generated using in-house PCRs (76.9%).
- Remaining results (23.1%) obtained by using commercially available kits.
- Most participants correctly characterized the vangene.
- After further molecular characterization of VRE13-07, the reported positive results are not due to the presence of part of the Tn1546 transposon.

Table 1. Summary of EQA results on all positive samples.

Sample code	Content	conc (CFU/ml)	Vancomycine Resistance status	vanGene	Total datasets n=39		PCR							
							Conventional				Real-time			
							Commercial (n=2)		In-house (n=7)		Commercial (n=8)		In-house (n=22)	
					n	%	n	%	n	%	n	%	n	%
VRE13-02	*E. faecium* IOWA 1	1,0.10^5	Positive	vanA	38	97,4	2	100	7	100	8	100	21	95,5
VRE13-11	*E. faecium* IOWA 2	1,0.10^5	Positive	vanB	32	82,1	2	100	3	42,9	6	75	21	95,5
VRE13-13	*E. faecium* LMG16165	1,0.10^7	Positive	vanA	38	97,4	2	100	7	100	8	100	21	95,5
VRE13-10	*E. faecium* LMG16165	1,0.10^6	Positive	vanA	36	92,3	2	100	7	100	7	87,5	20	90,9
VRE13-01	*E. faecium* LMG16165	1,0.10^5	Positive	vanA	36	92,3	2	100	6	85,7	7	87,5	21	95,5
VRE13-05	*E. faecalis* ATCC51299	1,0.10^7	Positive	vanB	32	82,1	2	100	3	42,9	6	75	21	95,5
VRE13-08	*E. faecalis* ATCC51299	1,0.10^6	Positive	vanB	34	87,2	2	100	4	57,1	6	75	22	100
VRE13-14	*E. faecalis* ATCC51299	1,0.10^5	Positive	vanB	28	71,8	1	50	4	57,1	6	75	17	77,3
VRE13-09	*E. gallinarum* ENT20120142	1,0.10^6	Positive	vanB+vanC	34	87,2	2	100	5	71,4	6	75	21	95,5
VRE13-06	*E. faecium* ENT20130036+ *E. gallinarum* LMG16289	1,0.10^5 1,0.10^5	Positive	vanC	14	35,9	1	50	3	42,9	2	25	8	36,4

Table 2. Characterisation of vanA operon ENT20130036.

			PCR-results									
strain nr	Origin	extraction method	vanA long (1013bp)	vanA Reflab (792bp)	orf1	orf2	vanR	vanS	vanH	vanX	vanYZ	IS1251
LMG16165	PC strain	EasyMAG	+	+	+	+	+	+	+	+	+	+
LMG16165	PC strain	HighPure Plasmid Kit	+	+	+	+	+	+	+	+	+	+
VRE13-07	clinical isolate	EasyMAG	-	-	-	-	-	-	-	-	-	-
VRE13-07	clinical isolate	HighPure Plasmid Kit	-	-	-	-	-	-	-	-	-	-
VRE13-07	QCMD panel	EasyMAG	-	-	-	-	-	-	-	-	-	-
MA076748	Gagnon et al	HiSpeed Plasmid mini kit	+	NA	-	-	+	+	+	+	+	+
MA076379	Gagnon et al	HiSpeed Plasmid mini kit	+	NA	-	-	-	-	+	+	+	+
MA075606	Gagnon et al	HiSpeed Plasmid mini kit	+	NA	-	-	-	-	+	+	+	+
MA081236	Gagnon et al	HiSpeed Plasmid mini kit	+	NA	-	-	-	-	+	+	+	+
MA081654	Gagnon et al	HiSpeed Plasmid mini kit	+	NA	-	-	-	-	+	+	+	+
MA080047	Gagnon et al	HiSpeed Plasmid mini kit	+	NA	-	-	-	-	+	+	+	+
MA077676	Gagnon et al	HiSpeed Plasmid mini kit	+	NA	-	-	-	-	+	+	+	+
VVE outbreak (n=30)	Szakacs et al	Qiaprep spin mini kit	+	NA	-	-	-	-	+	+	+	+