GGD Amsterdam

Improved diagnostics of vancomycin resistant enterococci by PCR on direct patient samples

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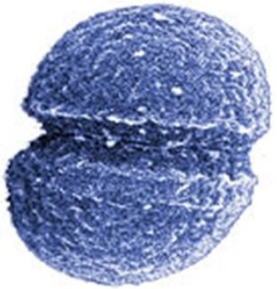


Background

- Enterococci were the first to acquire vancomycin resistance

- Vancomycin resistant enterococci (VRE): 3 main genotypes:
  vanA, vanB and *vanC;* genes code for glycopeptides
  VanA phenotype: resistance to vancomycin and teicoplanin
  VanB phenotype: resistance to vancomycin

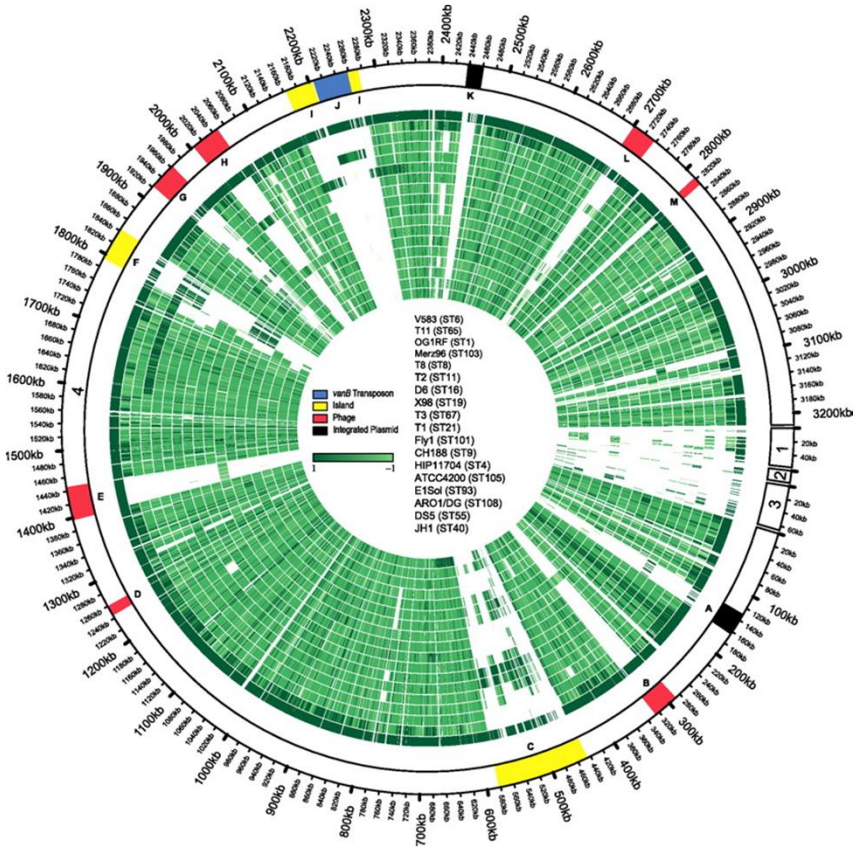
- Operons on plasmids or integrated in bacterial genome; transfer between different enterococcal species

- Primarily detected in *Enterococcus faecium*
  detection by culture takes 2 to 5 days

- VRE emerged as nosocomial pathogens

Aim of study

To improve VRE diagnostics by using PCR on patient samples or broths



Methods (culture)

- Retrospective study:
  inclusion of 53 frozen VRE strains from OLVG hospital

- Re-culturing and antibiogram determination
  E. faecium : furazolidone R and muporicin S
  VRE: vancomycin R

- Prospective study:
  inclusion of 175 swab samples. Both culture and o/n broth

- DNA extraction from 0.5 McFarland suspension (re-culture) or from o/n broth, using isopropanol precipitation method

Methods (molecular assays)

- Duplex PCR with Taqman probes for *vanA* and *vanB* genes from literature; Ct ≤ 36 is positive

- *AtpA* household gene: specific sequence for *Enterococci*
  Primers (from: F. Haagen, Nijmegen):
  Efa2-atpA-F 105: 5'-AGGTATCATTATCCTTGGCGATT-3'
  Efa2-atpA-R 2 : 5'-CGWCCYATCAAGGCCTCTC-3'

- Detection with SybrGreen melting curve analysis;
  Ct ≤ 36 is positive

Results retrospective study (1)

- VRE by culture 50 of 53 after re-culture
- VRE by PCR: positive for *atpA* and *vanA/vanB*

  50 of 53 detected, not all concordant with culture

		Culture antibiogram					VRE	PCR			
Antibiotic profile	Number of samples	GEN	AMP	NIT	FUR	MUP	VAN	*atpA*	*vanA*	*vanB*	VRE
Profile A	27	R	R	R	R	S	R	pos	pos	neg	yes
Profile A	1	R	R	R	R	S	R	pos	neg	neg	no
Profile A	1	R	R	R	R	S	R	pos	pos	pos	yes
Profile B	16	R	R	S	R	S	R	pos	pos	neg	yes
Profile C	2	Low R	R	S	R	S	R	pos	pos	neg	yes
Profile D	1	Low R	R	R	R	S	R	pos	pos	neg	yes
Profile D	1	Low R	R	R	R	S	R	pos	pos	pos	yes
Profile E	1	R	S	S	R	R	S	neg	neg	neg	no
Profile F	1	R	R	R	R	S	S	pos	pos	neg	yes
Profile F	1	R	R	R	R	S	S	pos	neg	neg	no
Profile G	1	R	S	R	R	S	R	pos	pos	neg	yes

Results retrospective study (2)

- Concordance of VRE detection by culture versus by PCR
  →all 53 samples were selected as VRE;
  2 were non-VRE upon re-culture

		CULTURE VRE	
		POS	NEG
PCR *atpA + vanA and /or vanB*	POS	49	1
	NEG	1	2
		Sensitivity	98%
		Specificity	67%

- Two discordant samples:
  -PCR pos/culture neg
  -PCR neg/culture VRE

Results prospective study

- Suspected VRE outbreak at OLVG hospital in May-June 2014
- Inclusion of 175 samples. Culture and PCR on o/n broth

		PCR				Culture	
Prospective profiles	Number of samples	atpA	vanA	vanB	VRE	OLVG	PHL
Profile H	4	pos	pos	neg	yes	VRE	-
Profile H	1	pos	pos	neg	yes	-	E. faecium
Profile I	7	pos	neg	pos	yes	-	E. faecium
Profile I	11	pos	neg	pos	yes	-	-
Profile J	18	pos	neg	neg	no	-	E. faecium
Profile J	44	pos	neg	neg	no	-	-
Profile J	5	pos	neg	neg	no	-	No growth
Profile K	1	neg	neg	neg	no	-	E. faecium
Profile K	61	neg	neg	neg	no	-	-
Profile K	11	neg	neg	neg	no	-	No growth
Profile L	1	neg	neg	pos	no	-	E. faecium
Profile L	10	neg	neg	pos	no	-	-
Profile L	1	neg	neg	pos	no	-	No growth

Results prospective study (2)

- Concordance of VRE detection by culture versus by PCR:
  19 discordant samples; 1 of 19 was *atpA* and *vanA* pos
  18 of 19 were *atpA* and *vanB* pos

		CULTURE VRE			
		POS	NEG		
PCR	POS	4	19	PPV	17%
atpA + vanA and /or vanB	NEG	0	152	NPV	100%
		Sensitivity	100%		
		Specificity	87%		

- Specificity is low (87%) and PPV (17%) is very low
  → due to *vanB* positives
- Re-calculation with *atpA* + *vanA* positive versus culture:
  specificity is 99% and PPV is now 80%

		CULTURE			
		POS	NEG		
PCR	POS	4	1	PPV	80%
atpA + vanA	NEG	0	170	NPV	100%
		Sensitivity	100%		
		Specificity	99%		

Discussion

- Negative results available within 24 hours
  if PCR testing is performed on overnight broth on a daily basis.

- Adding the *atpA* target to the *vanA* and *vanB* targets increases the negative predictive value.

- Specificity and PPV should be improved. Due to either:
  -false positive *vanB* results in 18/175 (10%) cases (*Clostridium spp ?*)
  In 15/18 cases the Ct value was >26: PPV could be 86%
  -false negative results in culture: two co-circulating VRE types

- Positive VRE need to be typed by MLST to establish if there is a clonal outbreak. See: www.efaecium.mlst.net
  This will support the actions to reduce transmission of VRE.

Conclusions

1. PCR on o/n broth shortens time to result compared to culture methods
2. VRE positivity may be excluded within 24 hours (NPV was 100%)

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