

Epidemiological and molecular characterization of the hospital-associated outbreak with vancomycin-resistant enterococci circulated in the Copenhagen area during 2013-2014 for a single hospital

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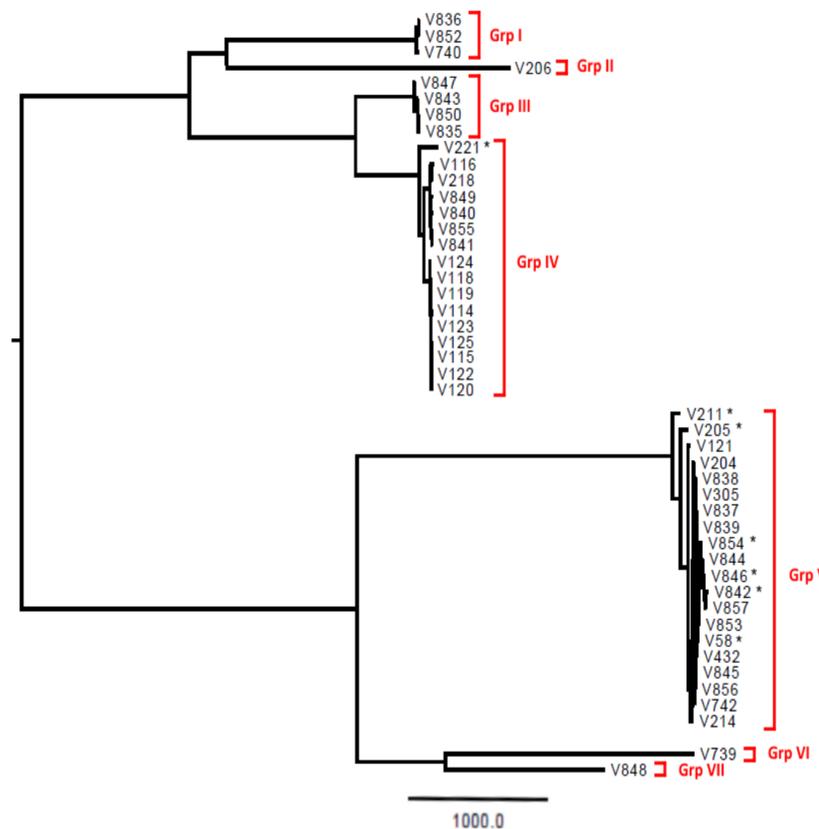
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Introduction: Vancomycin-resistant *Enterococcus faecium* (VRE) is a nosocomial pathogen, frequently associated with multidrug resistance. Typing methods used for outbreak investigation is an essential tool in determining bacterial relatedness.

Objectives: Hospital-associated outbreak with vancomycin-resistant *Enterococcus faecium* has been previously reported in the Copenhagen area. We would like to describe the situation with the VRE in a single hospital during the last 3½ years and present the results of 2 different molecular methods, which were used to define the similarity between the isolates.

Methods: Species identification was done by MALDI-TOF. Susceptibility testing for vancomycin was performed using Etest (BioMerieux) and interpreted according to the recommendation by EUCAST and confirmed by VanA gene PCR at the Reference laboratory.

Figure 1. Distribution of the vancomycin-resistant *E. faecium* based on the results for whole-genome sequencing.



*Pattern for ribotyping is not available.

Selected isolates were sequenced on the Illumina MiSeq platform. 2 x 150 bp paired-end-reads were produced. Reads were mapped with Stampy to a reference genome (AUS004_NC-017022).

Figure 2. Results for ribotyping combined with epidemiological data for *E. faecium* isolates.

N#	RiboGroup	RiboPrint™ Pattern				Department	Date of isolation	WGS Group
		1 kbp	5	10	15 50			
V836	ECORI 199-97-S-7					3114	02/11/2013	Grp I
V852	ECORI 199-97-S-7					3114RK1	21/11/2013	
V740	ECORI 199-97-S-7					2114GUL	03/02/2014	
V206	ECORI 199-1004-S-6					3074F1	04/04/2013	Grp II
V847	ECORI 199-97-S-7					4033L1	09/09/2014	
V843	ECORI 199-1069-S-1					3161ALLO	07/07/2014	Grp III
V850	ECORI 199-97-S-7					4131AN1	04/06/2014	
V835	ECORI 199-97-S-7					5052L1	03/04/2014	
V116	ECORI 199-97-S-7					3143B1	23/05/2013	Grp IV
V849	ECORI 199-97-S-7					4043L1	02/08/2014	
V840	ECORI 199-97-S-7					3121C1	14/05/2014	
V855	ECORI 199-420-S-1					3193TC8	06/07/2014	Grp V
V841	ECORI 199-97-S-7					4131	07/06/2014	
V124	ECORI 199-97-S-7					2164HAND	14/06/2013	
V118	ECORI 199-97-S-7					3193TC8	02/05/2013	Grp VI
V119	ECORI 199-97-S-7					5101FH10	16/03/2013	
V114	ECORI 199-97-S-7					4131AN1	17/03/2013	
V123	ECORI 199-97-S-7					4131AN1	24/04/2013	Grp VII
V125	ECORI 199-97-S-7					4131AN1	07/04/2013	
V115	ECORI 199-97-S-7					5121IR2	11/05/2013	
V122	ECORI 199-97-S-7					4131AN1	30/03/2013	Grp I
V120	ECORI 199-97-S-7					4131AN1	07/04/2013	
V121	ECORI 199-97-S-7					3153R0D	22/05/2013	
V204	ECORI 199-97-S-7					3124CA2	06/03/2013	Grp II
V838	ECORI 199-1033-S-8					3124CA2	12/11/2013	
V305	ECORI 199-97-S-7					3121C1	24/01/2013	
V837	ECORI 199-97-S-7					3114RK1	18/03/2014	Grp III
V839	ECORI 199-97-S-7					3121C1	09/06/2014	
V844	ECORI 199-97-S-7					4131AN1	22/08/2013	
V857	ECORI 199-420-S-1					2124C2	26/02/2014	Grp IV
V853	ECORI 199-97-S-7					3122C1	05/07/2014	
V432	ECORI 199-101-S-1					9301	13/01/2014	
V845	ECORI 199-1043-S-5					5052L1	01/12/2013	Grp V
V856	ECORI 199-97-S-7					3094NK1	12/07/2013	
V742	ECORI 199-97-S-7					4131AN1	16/09/2013	
V214	ECORI 199-1030-S-1					3124CA2	28/08/2013	Grp VI
V739	ECORI 199-1055-S-7					4131	04/05/2014	
V848	ECORI 199-97-S-7					4043	10/07/2014	

Ribotyping was performed on the selected isolates using the Riboprinter® microbial characterization system (DuPont Qualion) with the restriction enzyme EcoRI.

The analysis was performed according to manufacture precept and the analysis of the generated data was performed using the Riboprinter software.

The diversity of the samples were represented by cerebrospinal fluid (N=1); blood culture (N=4), urine culture (N=9); fluid from abdominal drain (N=4); tips of central venous catheter (N=5); swabs of various sample sides (N=4), as well as eleven other single isolates.

Dissemination of the isolates based on WGS and ribotyping is presented in the Figure 1 and 2. The results of WGS showed presence of 2 large (Group IV and Group V) and two small clones (Group I and III), as well as some sporadic isolates (Group II, Group VI, Group VII). In opposite, almost all isolates belong to a single type 97-S-7 due to the results of ribotyping. We did not found any consistence between these two methods.

Conclusions: Based on the WGS results there are two main VRE clonal types, causing the VRE outbreak. The results of ribotyping shows dissemination of one large VRE clonal type at Rigshospitalet in the period of investigation. Comparing the two molecular biology methods shows that WGS divides the VRE isolates in more clonal groups than ribotyping, which made it more discriminative.