Subtyping of vancomycin-resistant enterococci with MALDI-TOF mass spectrometry

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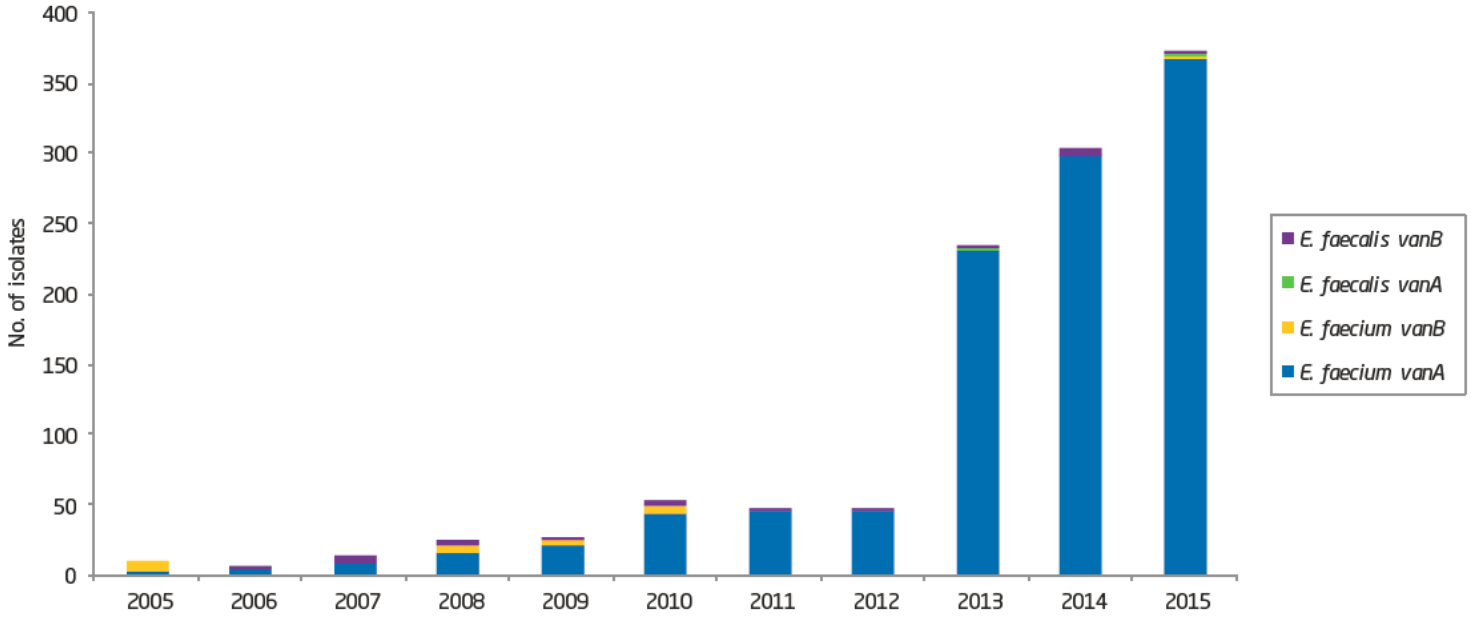
Background: increasing incidence of VREfm in Denmark

Figure 1. Numbers of vancomycin resistant *Enterococcus faecium* and *Enterococcus faecalis* isolates from clinical samples and van genes, Denmark DANMAP 2015



DANMAP 2015, http://www.danmap.org/downloads/reports.aspx

Molecular-based typing methods (PFGE, MLST, WGS):

time-consuming and costly

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Aim of the study

Usefulness of MALDI-TOF MS subtyping as first-line epidemiological tool?

Previous studies on MALDI-TOF MS subtyping of *E. faecium*: conflicting results

Differentiation of *vanA/B* positive versus negative *E. faecium*

Nakano et al. Int J Antimicrobial Ag 2014;44:256–259
Griffin et al. J Clin Microbiol 2012;50:2918-31

”Insufficient discriminatory power”

Lasch et al. J Microbiol Methods 2014;100:58-69

”Proof of concept”: discrimination hospital versus community associated strains

Freitas et al. Diagn Microbiol Infect Dis 2017;87:299–307

Small study comparing WGS-data with MALDI-TOF MS subtyping

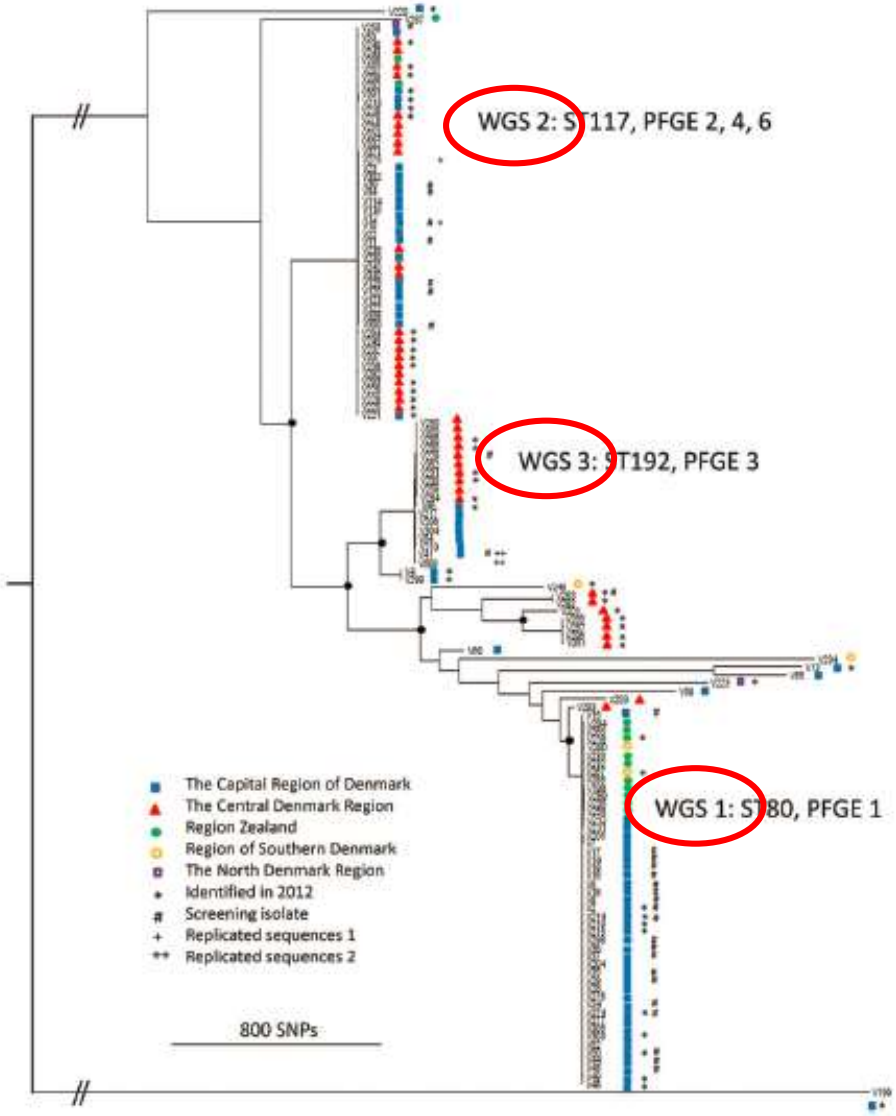
Schlebusch et al. Eur J Clin Microbiol Infect Dis 2017;36:495-499

Danish nationwide study:

132 *vanA* VREfm 2012/3 characterized by WGS

WGS I/II/III: n= 111 (84%)

non-WGS I/II/III (n=21)



Pinholt et al. J Antimicrob Chemother 2015 Sep;70(9):2474-82.

Study design

55 representative isolates:

18 WGS I (ST80)

11 WGS II (ST117)

9 WGS III (ST192)

17 non-WGS I/II/III (ST18, ST78, ST80, SLV-ST117, ST203, ST260, ST323, ST665)

Methods

Sample preparation

Ethanol-formic acid extraction as a triplet from the same 5% blood agar plate

3 spots per sample; 3 spectra per spot (Microflex LT; Bruker)

→ 27 spectra per isolate

Data analysis

baseline substraction, smoothening, internal calibration (6341,40 m/z)

Visual inspection in flexAnalysis to find discriminatory peaks

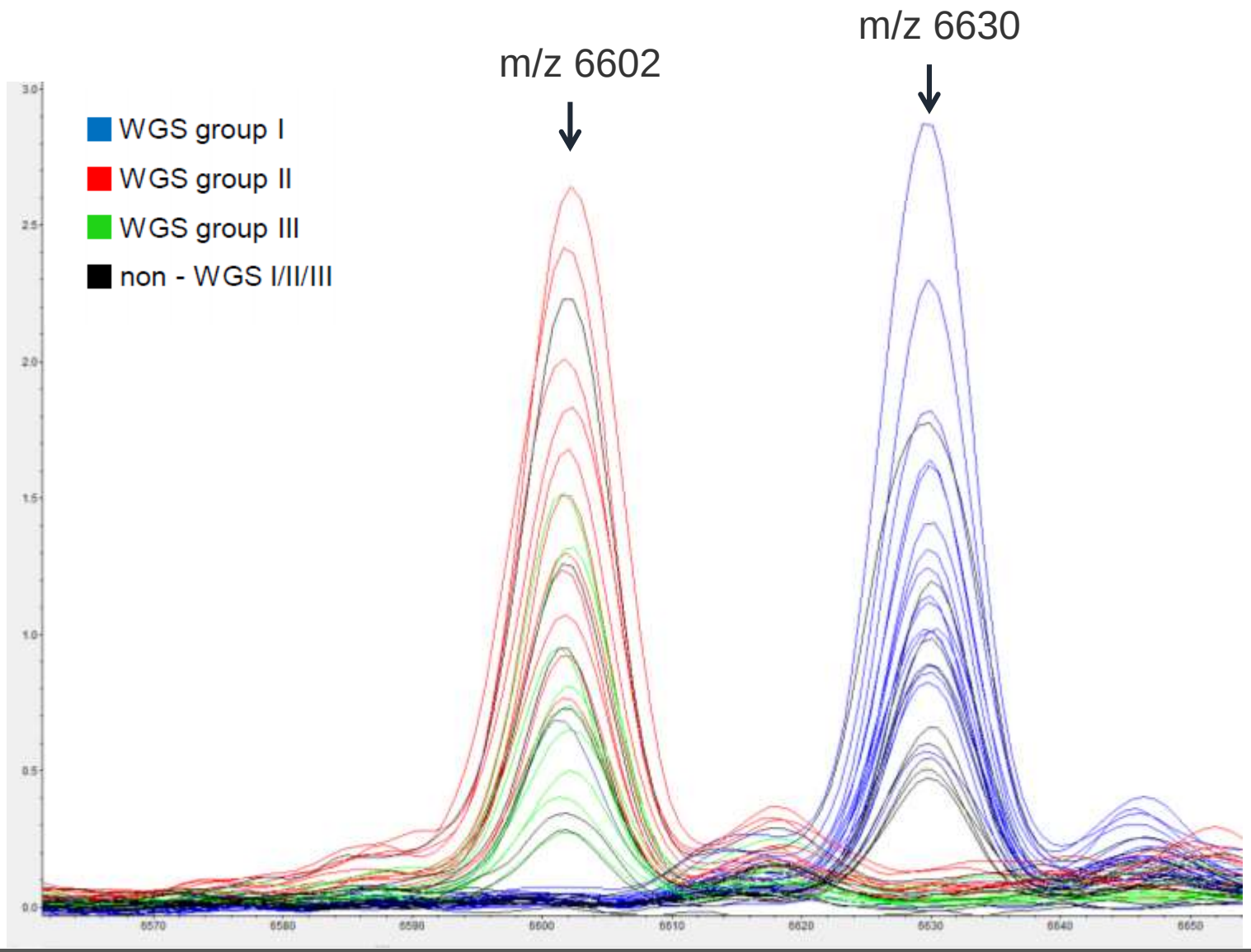
Definition of discriminatory peak

present in some, but not all isolates

well-demarcated bell-shaped peaks

consistent in all three extractions

unambiguous classification into “peak presence” or “absence” possible in all isolates


m/z 6602
m/z 6630
WGS group I
WGS group II
WGS group III
non - WGS I/II/III
3.0
2.5
2.0
1.5
1.0
0.5
0.0
6570
6580
6590
6600
6610
6620
6630
6640
6650

Results: peak scheme

Isolates belonging to the **WGS groups I-III** (n=38)

	WGS I																	WGS II											WGS III								
	ST80																	ST117											ST192								
m/z 3341	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
m/z 3924	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
m/z 5701	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
m/z 6354	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
m/z 6359	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
m/z 6602	-	-	(-)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
m/z 6630	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	(-)	(-)	(-)	(-)	(-)	-	-	-	-	(-)	-	-	-	-	-	-	-	-	-	-
m/z 6684	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
m/z 7848	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

↑ 1 / 38 misclassified

Results

Non-WGS I/II/III isolates (n=17)

	WGS I	WGS II	WGS III											non-WGS I/II/III							
	ST80	ST117	ST192		ST80			ST260	ST18					SLV ST117	ST78			ST665	ST203	ST323	
m/z 3341	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	
m/z 3924	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	
m/z 5701	-	+	-	-	+	+	-	-	-	-	-	+	+	+	-	-	-	+	-	-	
m/z 6354	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	
m/z 6359	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	
m/z 6602	-	+	+	+	-	-	(-)	-	-	-	-	-	-	+	+	+	+	+	+	-	
m/z 6630	+	-	-	-	+	+	+	+	+	+	+	+	+	(-)	-	-	-	-	-	-	
m/z 6684	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	
m/z 7848	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	

10 misclassified as WGS I, WGS II or WGS III

Conclusions

Subtyping method is more demanding than MALDI-TOF MS for routine species identification

Identification of 9 discriminatory peaks

→ reliable discrimination between WGS group I/II/III
→ BUT: misclassification of 10/17 non-WGS I/II/III

→ limited practical value for this method as first-line epidemiological tool for VREfm outbreak management

Thanks

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