



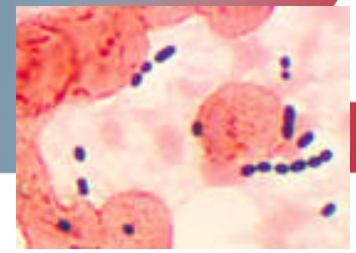
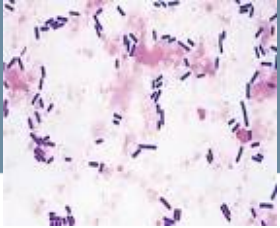
Surveillance of Enterococci in Belgium

M. Ieven, K. Loens, B. Jans and H. Goossens

Overview

- Introduction and epidemiological surveillance
- Results of isolates received at the NRC
- Outbreaks
- “(Re)-emerging” strains?

Enterococci: Introduction



- Enterococcus spp. is now the 3rd most common pathogen in hospitalized patients, accounting for +/- 12% of nosocomial infections including:
 - endocarditis, bloodstream infections, and urinary tract infections,
 - associated with peritonitis and intra abdominal abscesses.

Characteristic	Phenotype				
	VanA	VanB	VanC	VanD	VanE/G
Vancomycin MIC (µg/ml)	64->1000	4->1000	2-32	16-64	16
Teicoplanin MIC (µg/ml)	16-512	0.5 > 32	0.5-1	2-4	0.5
gene	<i>vanA</i>	<i>vanB</i>	<i>vanC1-C2</i>	<i>vanD</i>	<i>vanE</i>
frequent enterococcal species	<i>E. faecium</i> <i>E. faecalis</i>	<i>E. faecalis</i> <i>E. faecium</i>	<i>E. gallinarum</i> <i>E. casseliflavus</i> / <i>E. flavescens</i>	<i>E. faecium</i> <i>E. faecium</i>	<i>E. faecalis</i> <i>E. faecalis</i>
determinant	Acquired	Acquired	Intrinsic species characteristic	Acquired	Acquired
transferable	Yes	Yes	No	No	No

Epidemiological surveillance of resistant Enterococci 2015

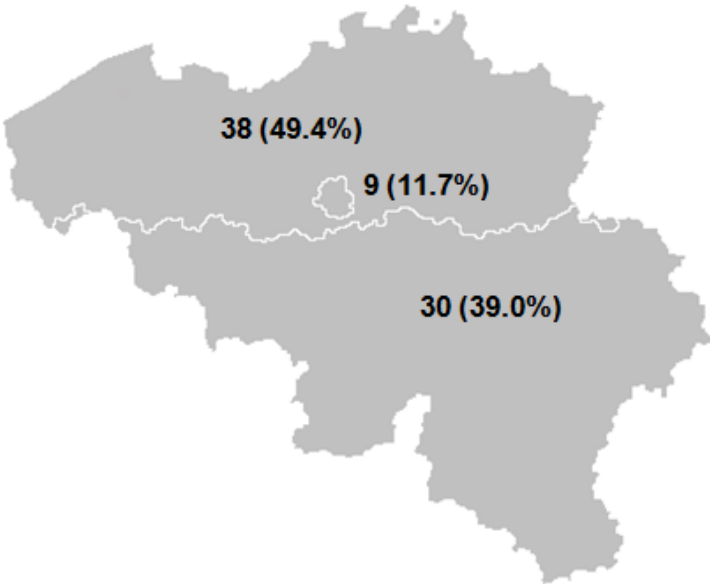
77 participating laboratories (28.590 beds, LoS: 7.3 d.)

Distribution by region

Region	n. hospitals	n. beds	mean LoS (d.)
Flanders:	38 (49.4%)	16105	7.0
Wallonia:	30 (39.0%)	9541	7.0
Brussels:	9 (11.7%)	2944	7.8

Distribution by hospital size

Beds	n. hospitals	n. beds	LoS (d.)
< 200 beds:	19 (24.7%)	2349	8.2
200 - 399 b.:	33 (42.9%)	9874	6.9
≥ 400 beds:	25 (32.5%)	16367	7.0

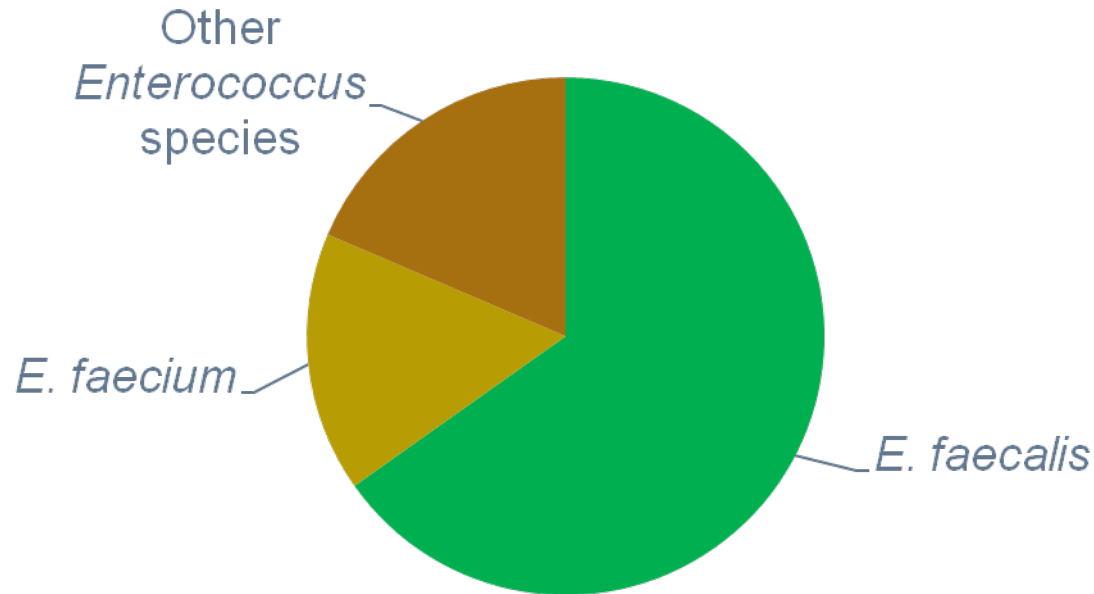


Laboratory practices

Applied criteria for CMI-breakpoints (EUCAST versus CLSI),
Routinely susceptibility testing for teicoplanine and for linezolid

Region	EUCAST	CLSI	testing teico-S	testing linezolid-S
Flanders	76%	24%	43%	50%
Walloon	80%	20%	87%	80%
Brussels	33%	67%	89%	75%
BELGIUM	73%	27%	66%	65%

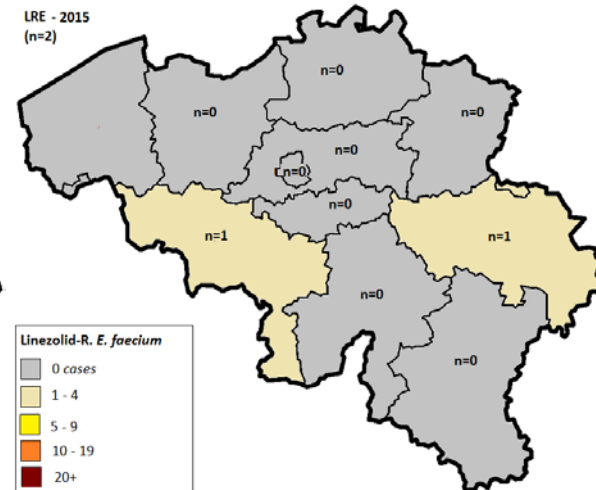
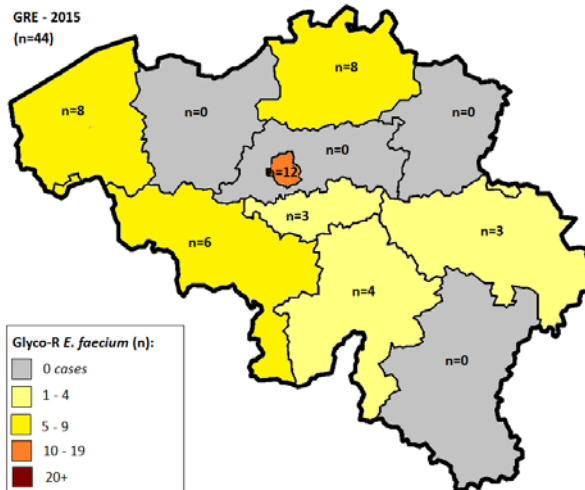
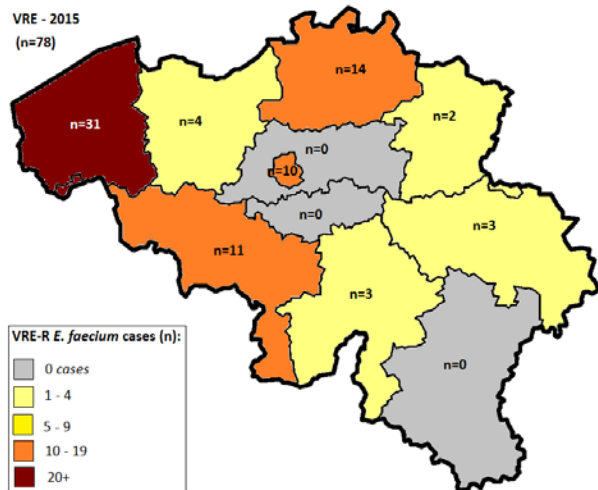
Total number of isolated *Enterococci* in 68 belgian laboratories 2015



	n	%
<i>Enterococcus faecalis</i>	13 094	65.2%
<i>Enterococcus faecium</i>	3 261	16.2%
Other <i>Enterococcus</i> species	3 730	18.6%
Total number of isolated ENTEROCOCCI	20 085	100%

Resistant *Enterococcus faecium* in Belgian hospital

2015

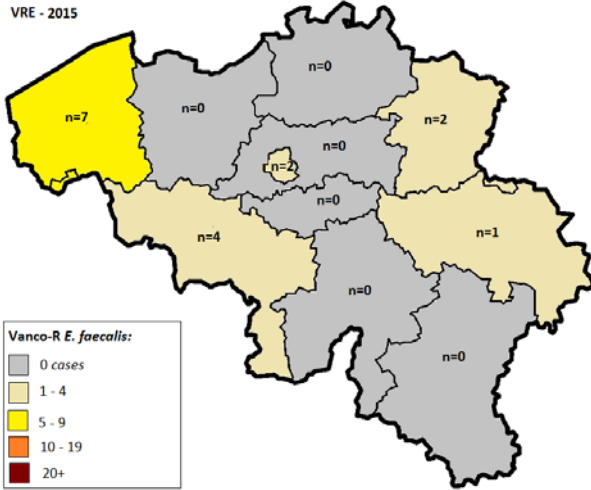


2015	vancomycine-R (n=78)		glycopeptide-R (n=44)		linezolid-R (n=2)	
	%	/1000 adm	%	/1000 adm	%	/1000 adm
Flanders	2.3%	0.09	1.4%	0.054	0.00%	0.000
Walloon	1.7%	0.05	1.8%	0.054	0.24%	0.007
Brussels	2.3%	0.10	4.8%	0.175	0.00%	0.000
Belgium	2.1%	0.08	1.9%	0.067	0.08%	0.003
2014	1.8%	0.06	3.0%	0.092	0.04%	0.002

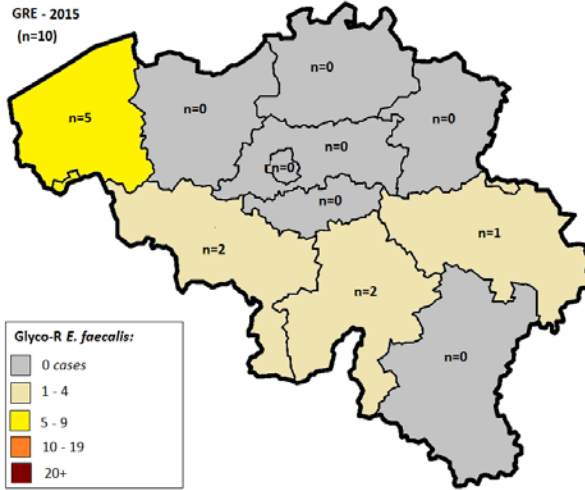
Resistant *Enterococcus faecalis* in Belgian hospital

2015

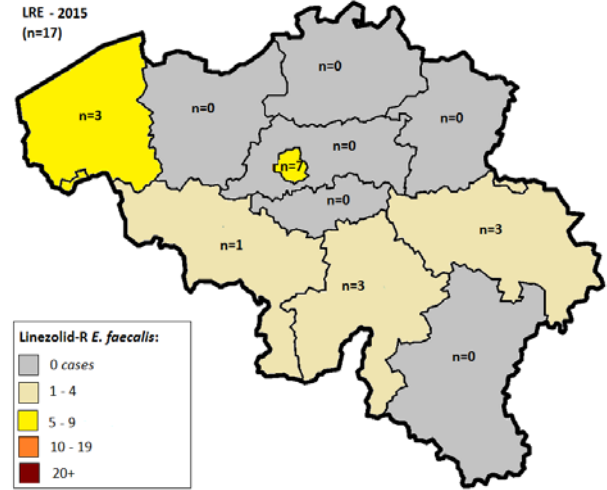
VRE - 2015



GRE - 2015
(n=10)



LRE - 2015
(n=17)



2015	vancomycine-R (n=16)		glycopeptide-R (n=10)		linezolid-R (n=17)	
	%	/1000 adm	%	/1000 adm	%	/1000 adm
Flanders	0.10%	0.015	0.11%	0.020	0.06%	0.010
Walloon	0.13%	0.015	1.14%	0.017	0.21%	0.026
Brussels	0.12%	0.020	0.0%	0.000	0.47%	0.074
Belgium	0.11%	0.016	0.11%	0.016	0.16%	0.025
2014	0.06%	0.009	0.11%	0.014	0.06%	0.008

Discussion: epidemiological surveillance

2014-2015:

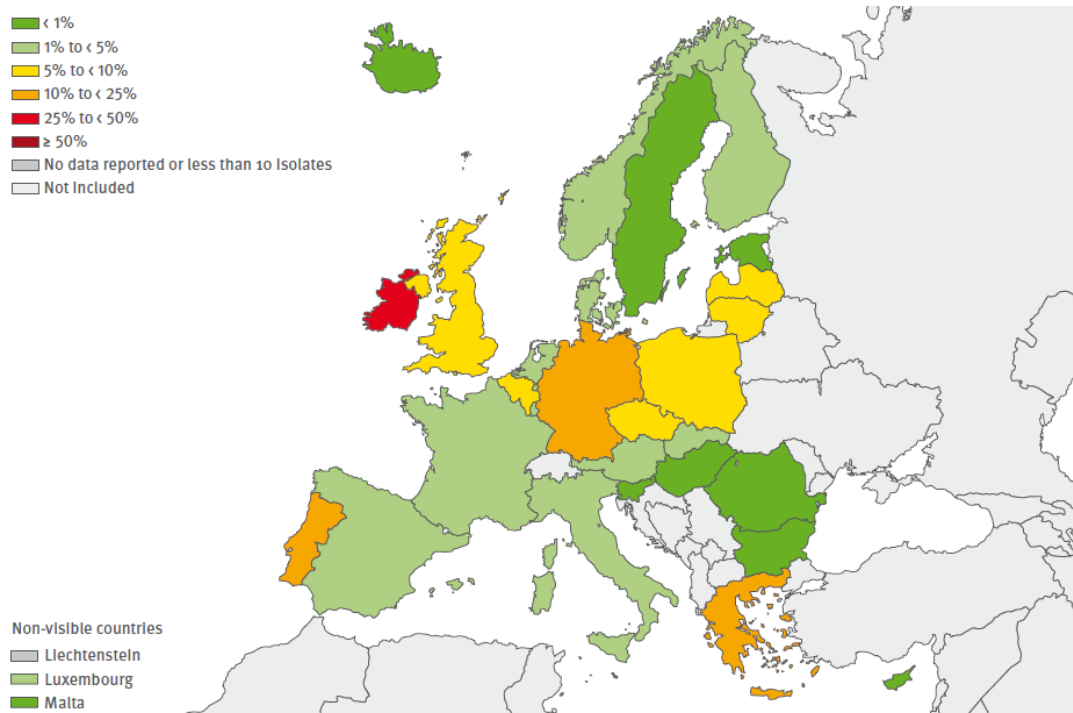
- increasing number of participating hospitals
- increasing % and incidence of vancomycin-R *E. faecium* and *E. faecalis*
- important increase of the proportion and incidence of linezolid-R *E. faecalis*:

% linezolid-R *E. faecalis*: **0.16%** (2015) versus **0.06%** (2014),
incidence of linezolid-R *E. faecalis*: **0.02/1000 adm** (2015)
versus **0.008/1000 adm** (2014)

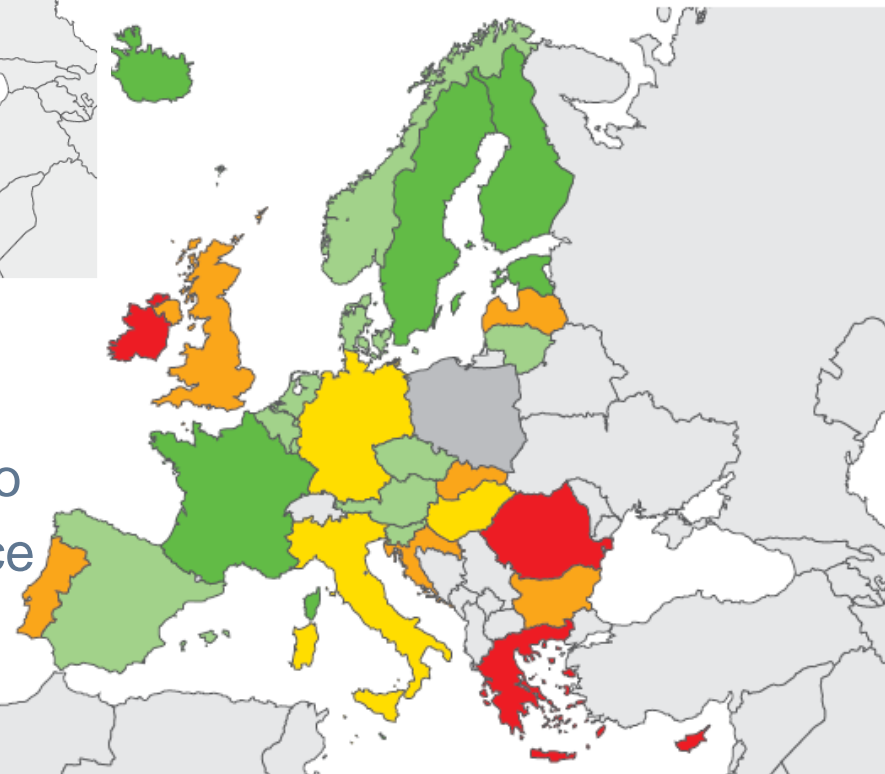
... **but these isolates should be confirmed by the NRC**

- increasing number of hospitals (n=8) with (large) outbreaks involving resistant *E. faecium*.

The VRE problem in Europe: *Enterococcus faecium*: % vanco R invasive isolates, by country, 2011 & 2014



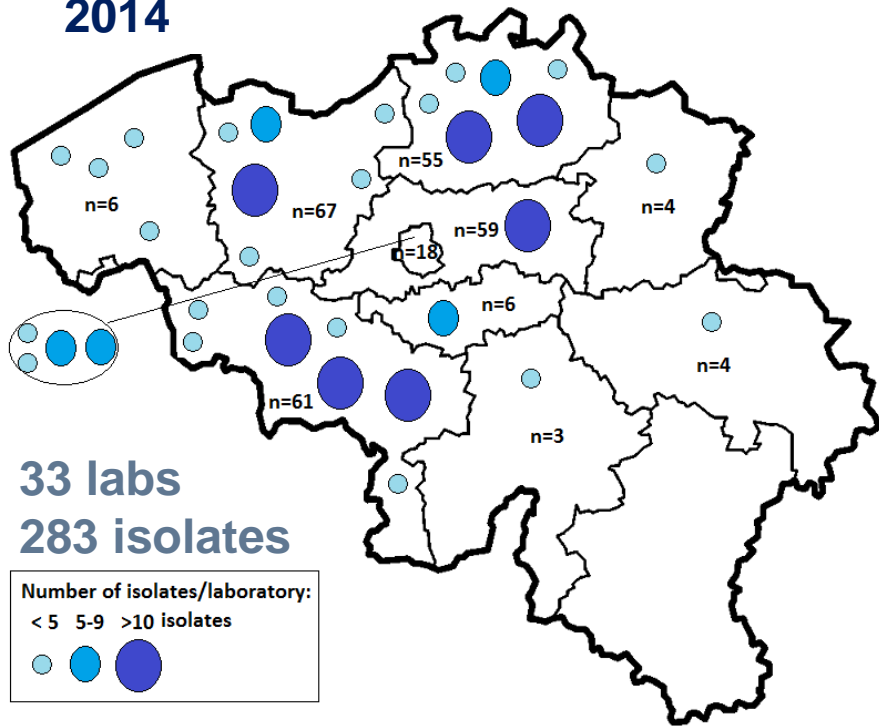
- The emergence of particular clones of *E. faecalis* and *E. faecium* was paralleled by increases in resistance to glycopeptides and high-level resistance to aminoglycosides,



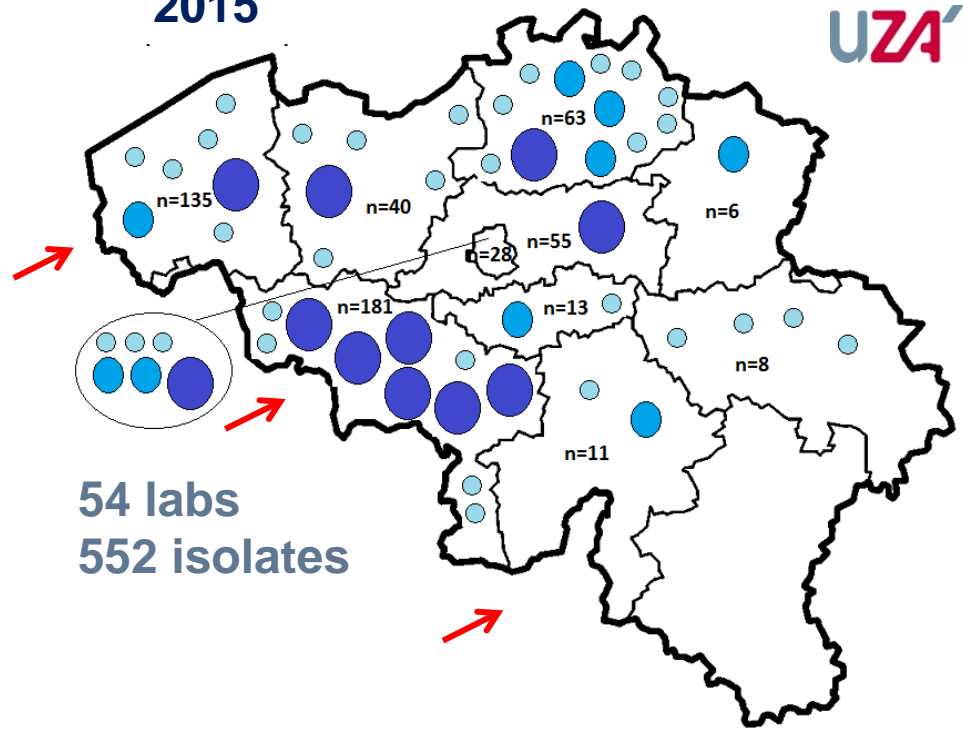
NRC: tests performed for Enterococci

- Confirmation of identification (routine)
 - *E. faecium*, *E. faecalis*, *E. casseliflavus*, *E. gallinarum* and *E. raffinosus*
- Confirmation of phenotypic susceptibility testing: on all isolates
 - MIC for ampicillin, vancomycin, teicoplanin, linezolid, tigecycline
 - HLR (gentamicin, streptomycin)
- Genotypic detection of resistance genes:
 - All species: if MIC $\geq 4 \Rightarrow$ PCR *vanA,B* (if neg: PCR *vanD,E,G*)
 - All *E. faecium* MIC $< 4 \Rightarrow$ PCR *vanA* to exclude VVE
 - All *E. gallinarum/E. casseliflavus* PCR *vanA,B,C*
- In case of outbreak investigation:
 - Pulsed Field Gel Electrophoresis (PFGE)
 - MultiLocus Sequence Type (MLST)

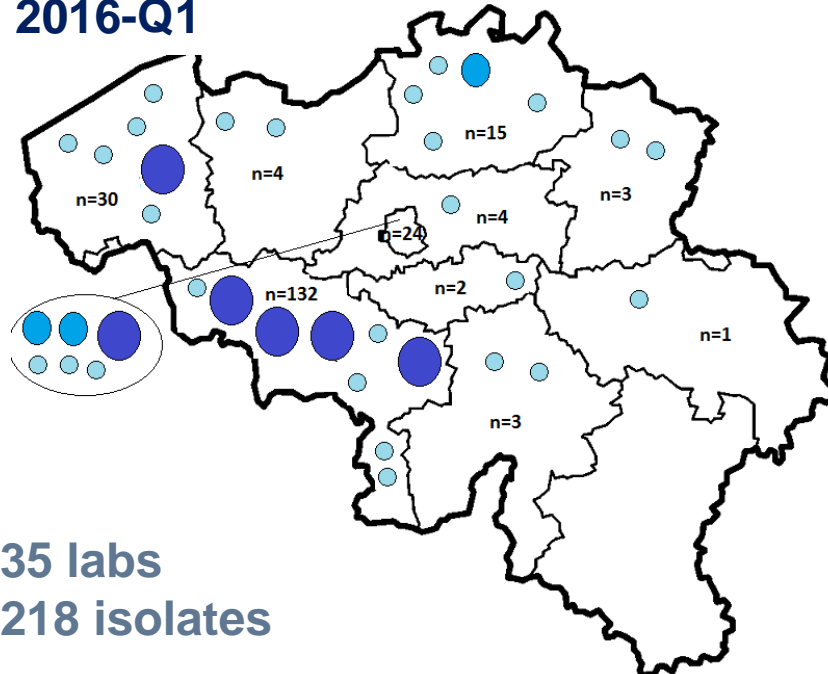
2014



2015



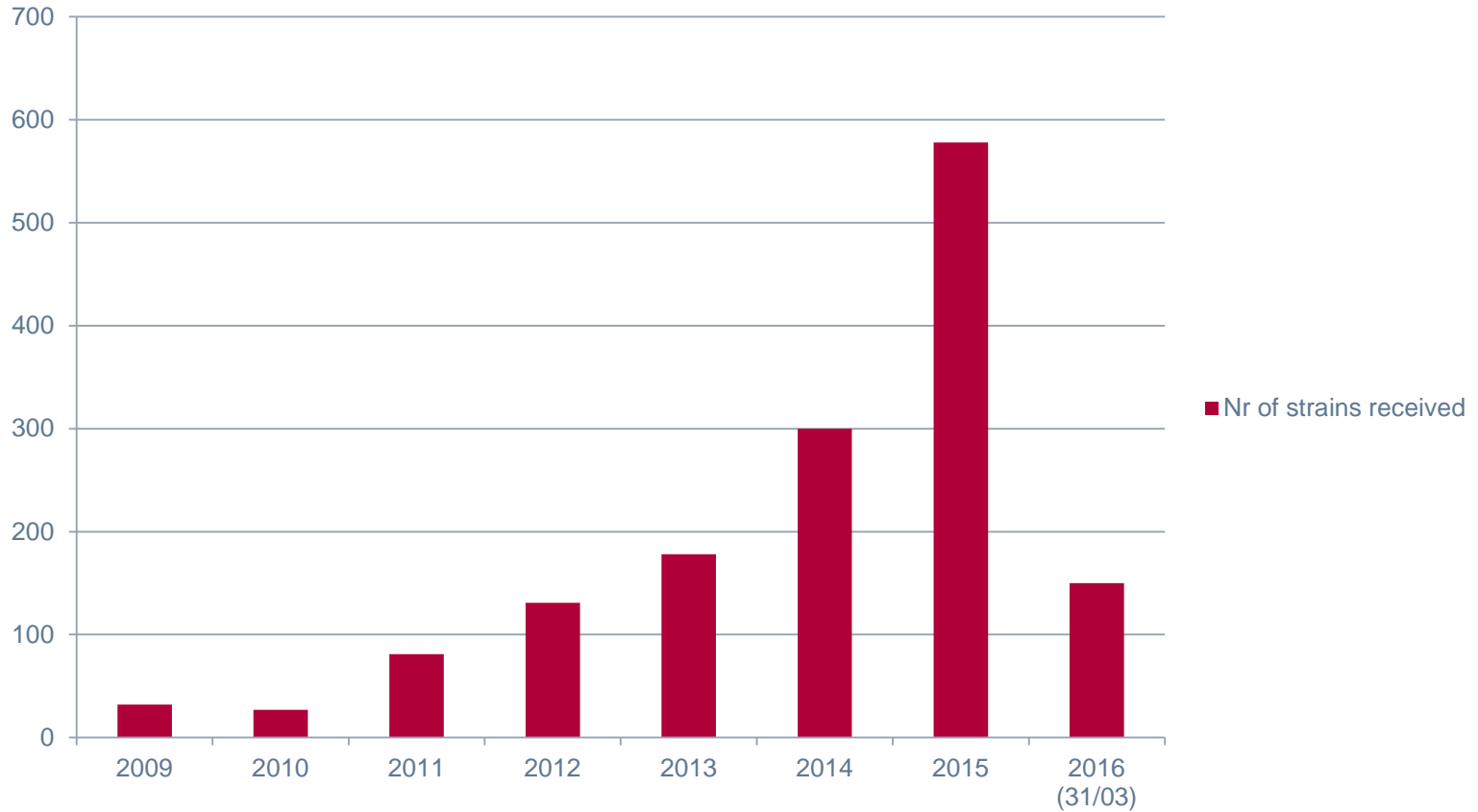
2016-Q1



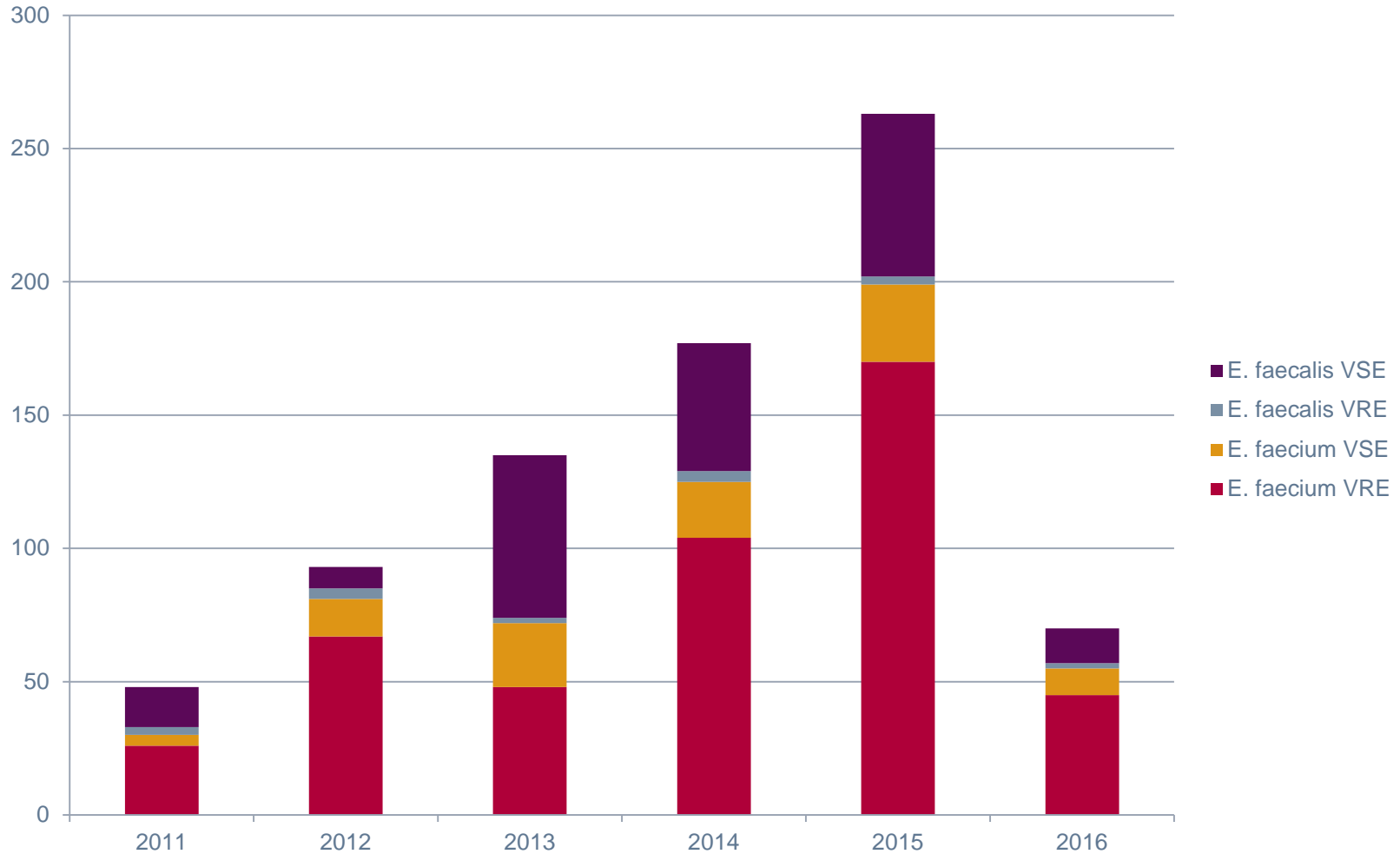
Number of participating laboratories and number of isolates by region

Overall nr of strains received: 01/01/2009-31/03/2016

Nr of strains received



Nr of enterococcal isolates from an infection

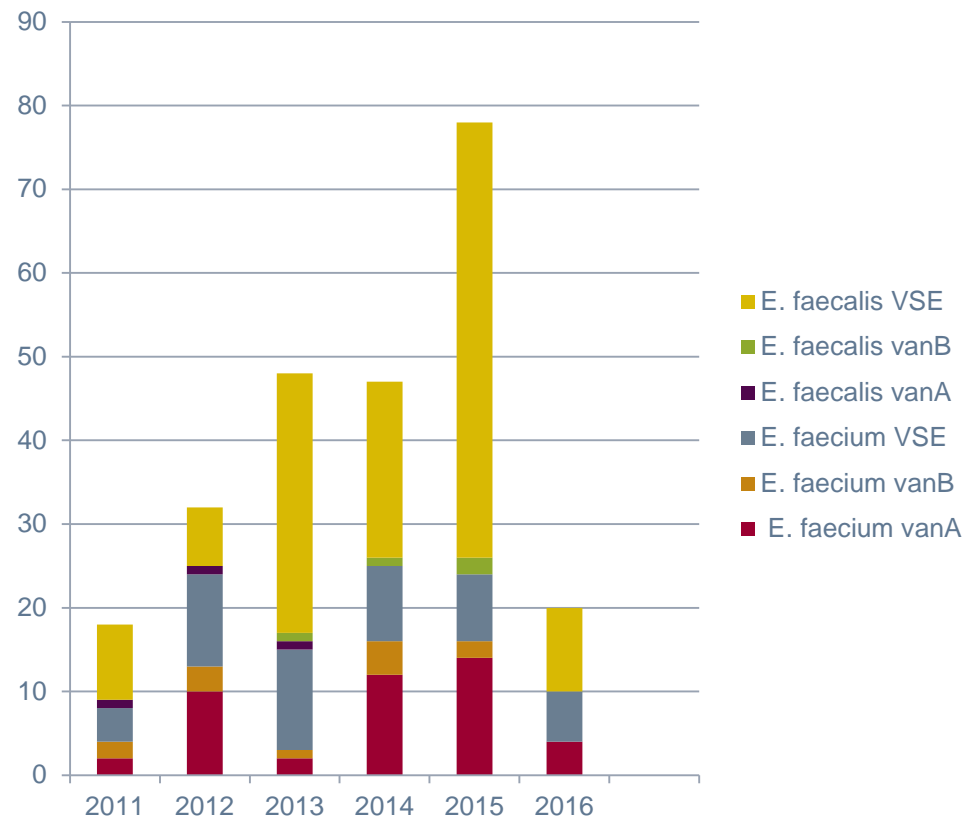


Relative importance of enterococci isolated from different infections

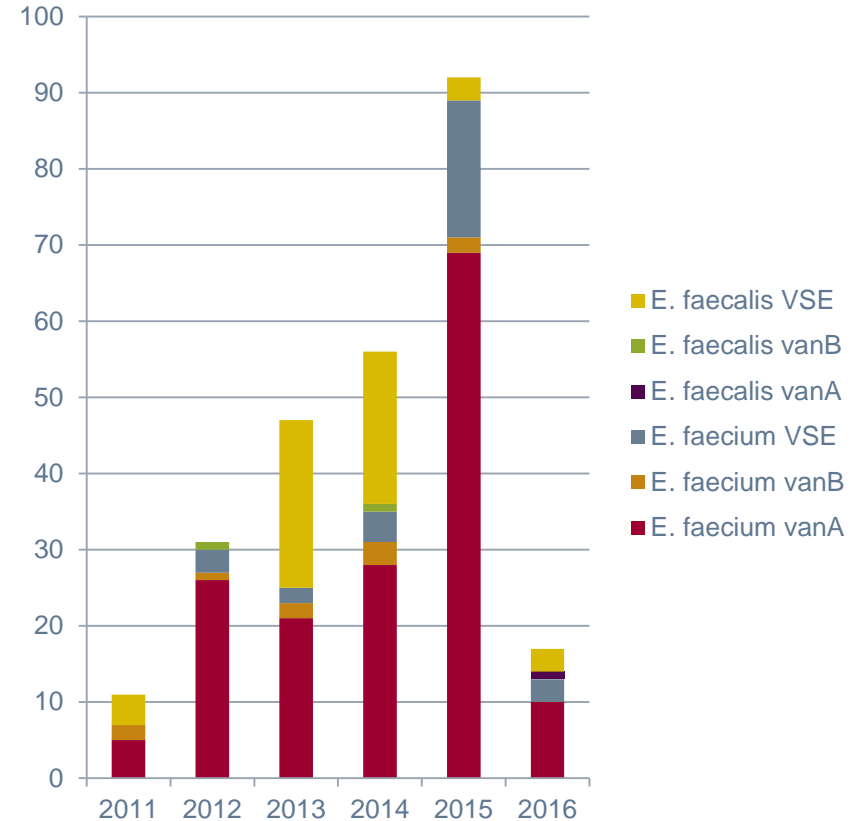
Year	2011	2012	2013	2014	2015	2016*	Total
Blood	20	36	54	49	79	20	258
Urine	13	31	51	61	94	15	265
(non)-sterile wound	5	16	27	18	39	18	123
Tissue	0	1	0	1	3	1	6
Catheter	2	2	0	5	1	2	12
Peritoneal	3	5	7	6	9	3	33
non-sterile	4	3	2	21	10	8	48
broncho-tracheal	5	1	0	1	8	1	16
Other sterile	7	7	11	1	15	4	45
Other	0	0	0	30	17	0	47
Total	59	102	152	193	275	72	853

- 679/853 (79.6%) of isolates from bacteremia, urinary tract- or wound infections, and peritonitis

E. faecium and *E. faecalis* from blood and urine



Blood isolates



Urine isolates

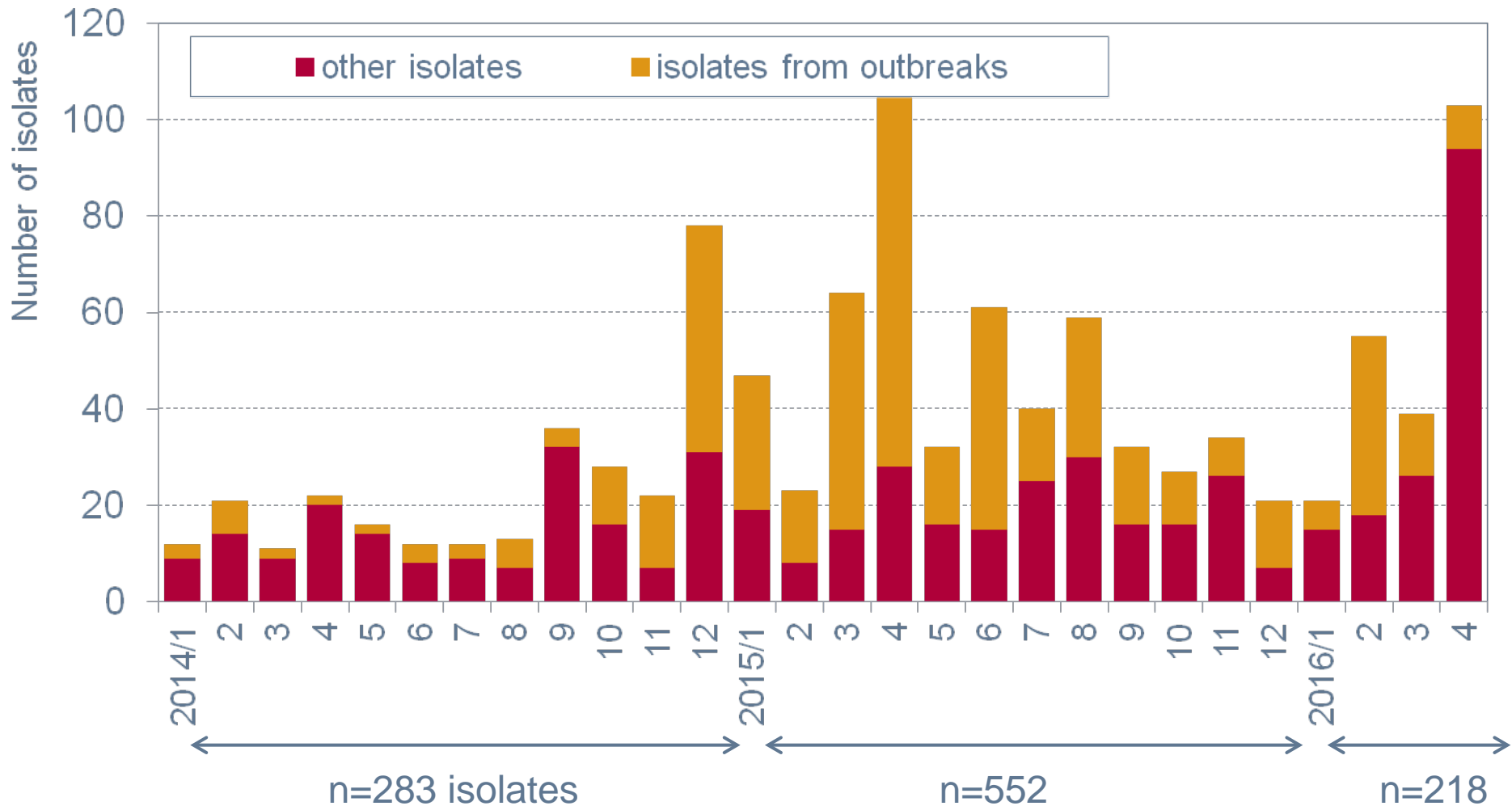
Enterococcal strains received at the NRC (01/01/2009-31/03/2016)

	2009	2010	2011	2012	2013	2014	2015	2016
Nr of strains received	32	27	81	131	178	300	578	150
UNK	0	4	2	0	0	0	0	0
<i>E. faecium</i>	18	19	47	104	95	215	447	132
<i>E. faecalis</i>	12	3	18	15	67	68	111	15
<i>E. cass/gall</i>	2	1	13	9	12	12	14	2
Other Ent. spp	0	0	1	3	4	4	4	1
Nr of VRE (%)	18 (56.3)	21 (77.8)	61 (75.3)	103 (77.4)	85 (47.8)	198 (65.8)	444 (76.1)	125 (83.3)
<i>vanA</i> (%)	5 (27.8)	8 (38.1)	24 (39.3)	84 (81.6)	61 (71.8) ↑	163 (82.3) ↑	402 (90.5) ↑	123 (98.4) ↑
<i>vanB</i> (%)	11 (61.1)	12 (57.1)	24 (39.3)	11 (10.7)	12 (14.1)	20 (10.1)	24 (5.4)	1 (0.8)
<i>vanC</i> (%)	2 (11.1)	1 (4.8)	13 (21.3)	8 (7.7)	12 (14.1)	11 (5.6)	13 (2.9)	1 (0.8)
Other (%)	0	0	0	0	0	3 (1.5)	5 (1.1)	0
Nr of outbreaks*	1 (n=6)	1 (n=3)	3 (n=4, 12, 16)	4 (n=3, 4, 6, 39)	1 (n=36)	10 (n=2, 3, 3, 3, 6, 6, 8, 14, 35, 46)	22** (n=2, 5x3, 4, 4, 5, 5, 6, 6, 9, 11, 12, 12, 15, 18, 20, 37, 54, 76)	

*All except 1 due to *E. faecium*; ** 1 clone in several hospitals in the same region; outbreak: if indicated by local lab and confirmed by NRC-typing

Monthly number of *Enterococcus* isolates

2014-2016 (Q1): 1053 isolates

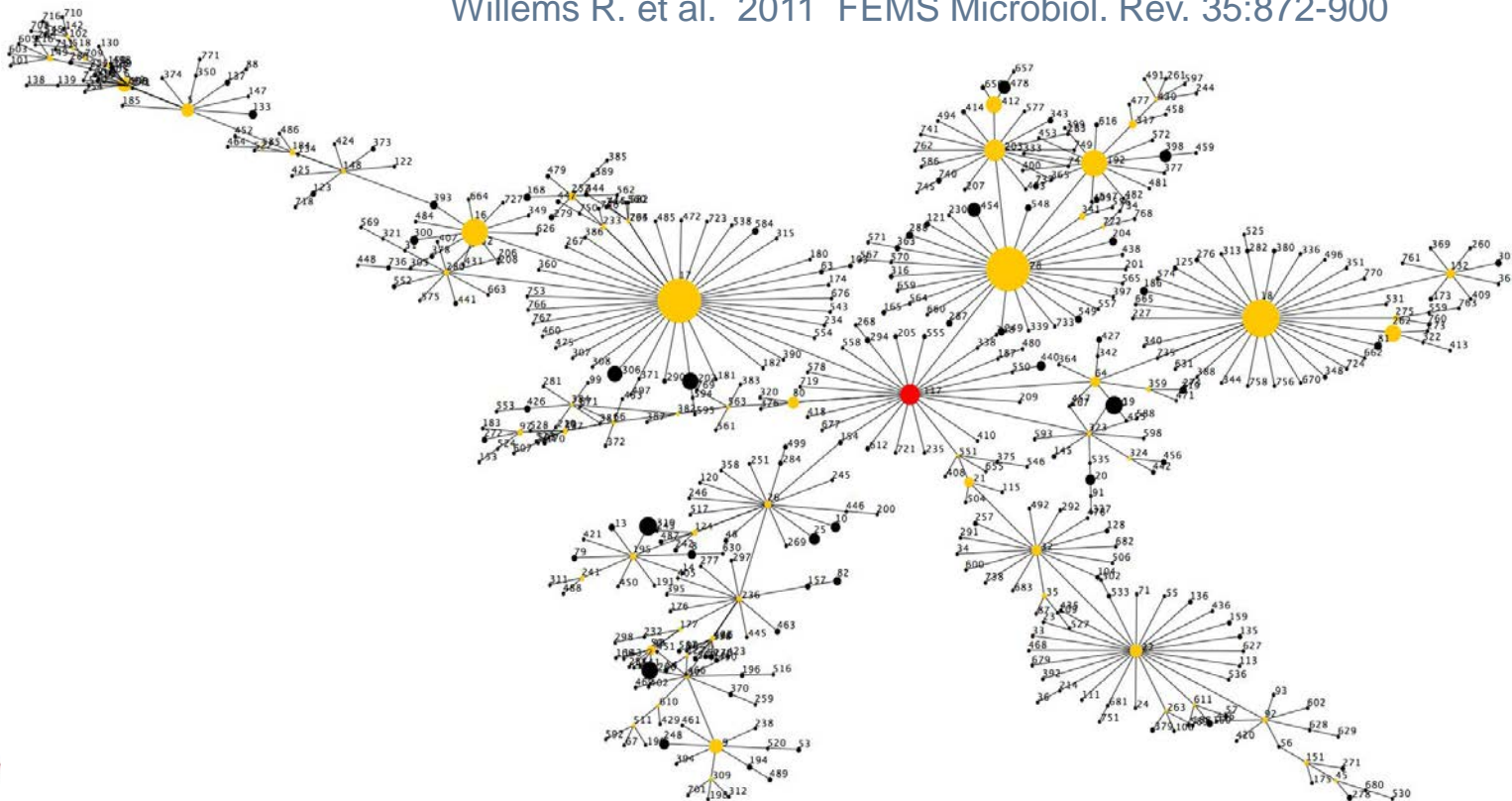


E. faecium Clonal Complex 17

- Multilocus sequence typing (MLST) studies of *E. faecium* revealed the existence of a distinct genetic subpopulation associated with nosocomial epidemics: **clonal complex 17 (CC17)**, exhibiting high-level vancomycin, ampicillin, and quinolone resistance on the basis of MLST data

Willems R. et al. 2005 Emerging Infect. Dis. 11:821-28

Willems R. et al. 2011 FEMS Microbiol. Rev. 35:872-900



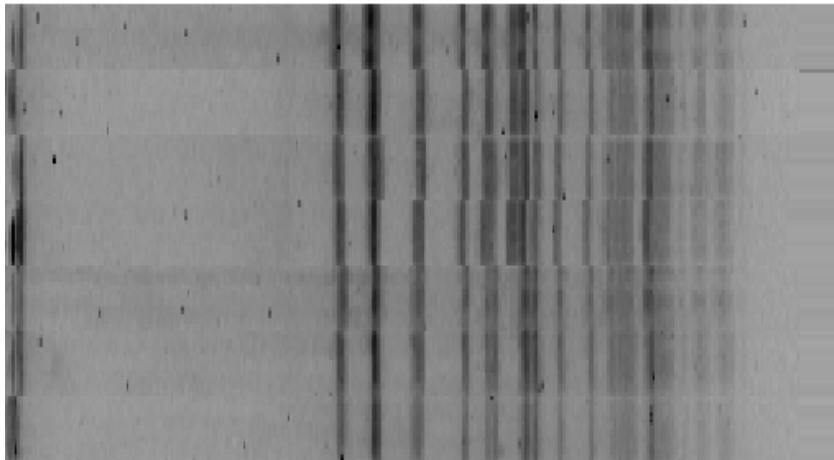
ST-types *E. faecium* outbreak vs non-outbreak strains

Done for 190 strains, selection based on outbreak/non-outbreak, geography, in function of time

ST-types	2012*	2013	2014	2015
Outbreak	ST203	ND	<u>ST18, 80, 117, 203</u>	<u>ST17, 117, 203</u>
Non-outbreak	ND	ND	ST78, <u>80, 192, 412, 413, 925</u>	ST16, 17, 18, 19, 78, <u>80, 117, 203, 290, 885, 925</u>
UNK	ND	ND	ST17, 18, <u>80, 117, 203, 577, 925</u>	ST17, 18, 78, <u>80, 117, 203, 612</u>

* Only retrospectively done for 1 outbreak, Coppens et al. Ready for submission

ST-types correlated to PFGE-typing



ENT2015/335

ENT2015/349

ENT2015/320

ENT2015/319

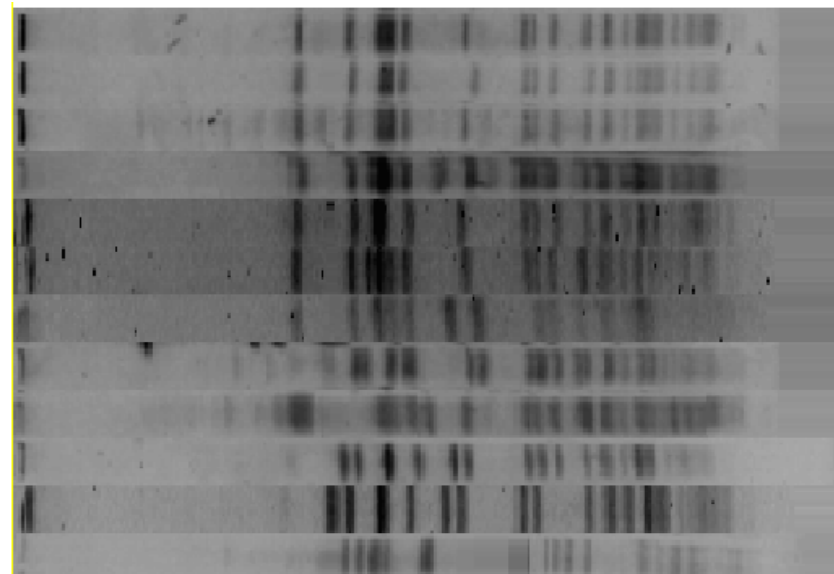
ENT2015/346

ENT2015/347

ENT2015/348

ST203, PFGE type 62,
Hospital 1, Hainaut region

ST203, PFGE type 62,
Hospital 2, Hainaut region



ENT 2014/209

ENT 2014/210

ENT 2014/208

ENT 2014/228

ENT2015/355

ENT2015/401

ENT2015/358

ENT 2014/205

ENT 2014/194

ENT 15/038

ENT2015/327

ENT2014/131

ST117, PFGE type 98, H3

ST117, PFGE type 98-2, H4

ST117, PFGE type 98, H3

ST117, PFGE type 98, H3

ST117, PFGE type 149, H5

ST117, PFGE type 112, H6

ST117, PFGE type 16, H7

ST117, PFGE type 6, H7

ST117, PFGE type 123, H7

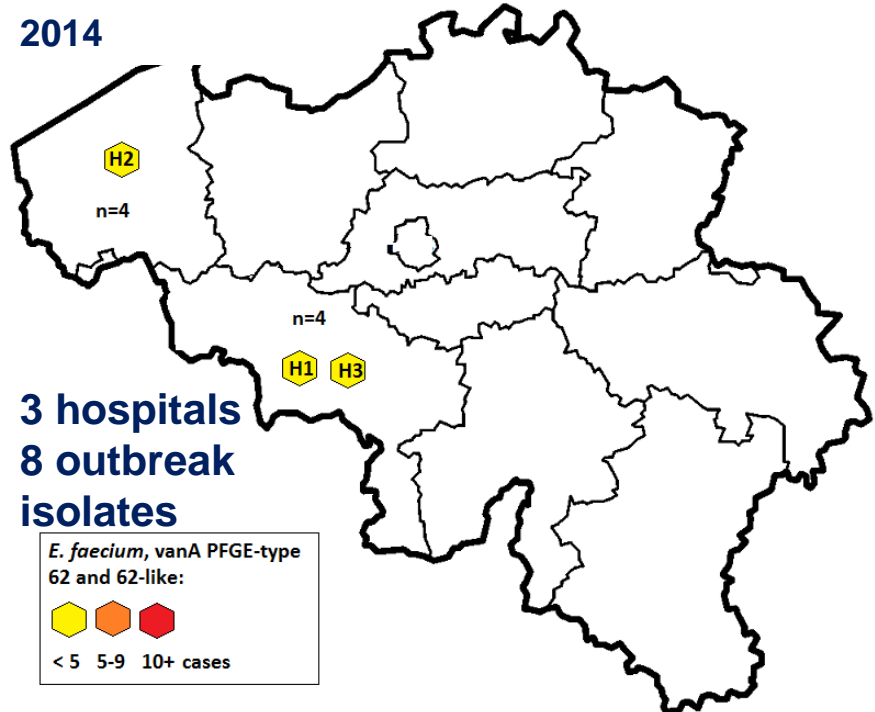
ST117, PFGE type 95, H1

Reported outbreaks involving resistant *Enterococci* in Belgian hospitals 2015

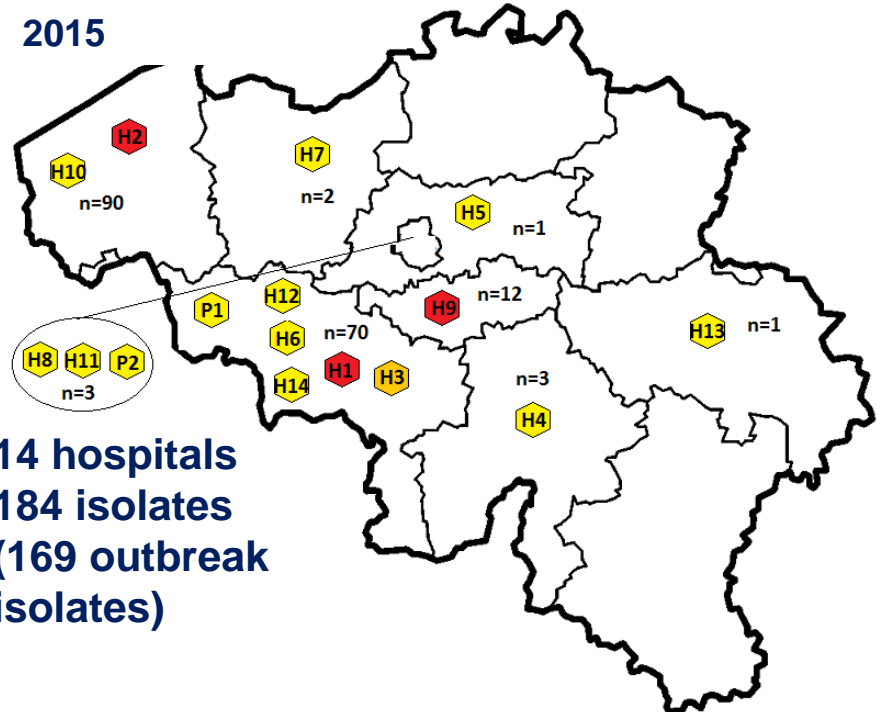
- 8 hospitals with ≥ 1 outbreak involving resistant Enterococci, on total 12 episodes
- 144 outbreak cases (min. 2, max. 86 cases/hospital) - 21 infected

Hospitals	Number of cases	Region	Species	Van genes	resistance	PFGE-type	MLST-type
Hosp A	86	Flanders	<i>E. faecium</i>	vanA	Vanco-R	62, 114, 115	203, 17, 80
Hosp B	9	Flanders	<i>E. faecium</i>	vanA	Vanco-R	62	203
Hosp C	15	Flanders	<i>E. faecium</i>	vanA	Vanco-R	101	80
Hosp D	4	Flanders	<i>E. faecium</i>	vanB	Vanco-R	196	203
Hosp E	2	Brussels	<i>E. faecium</i>	vanA	Vanco-R	123	80
Hosp F	7	Wallonia	<i>E. faecium</i>	vanA	Glyco-R	62	203
Hosp G	11	Wallonia	<i>E. faecium</i>	vanA	Glyco-R	62	203
Hosp H	10	Wallonia	<i>E. faecium</i>	-	Glyco-R	isolates not sent to the NRC	

2014

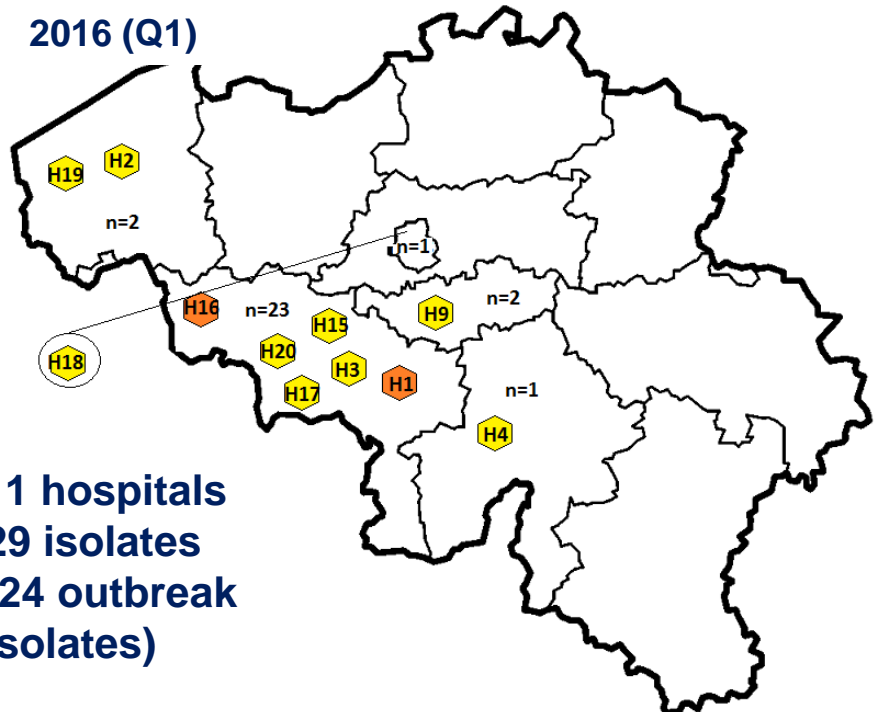


2015

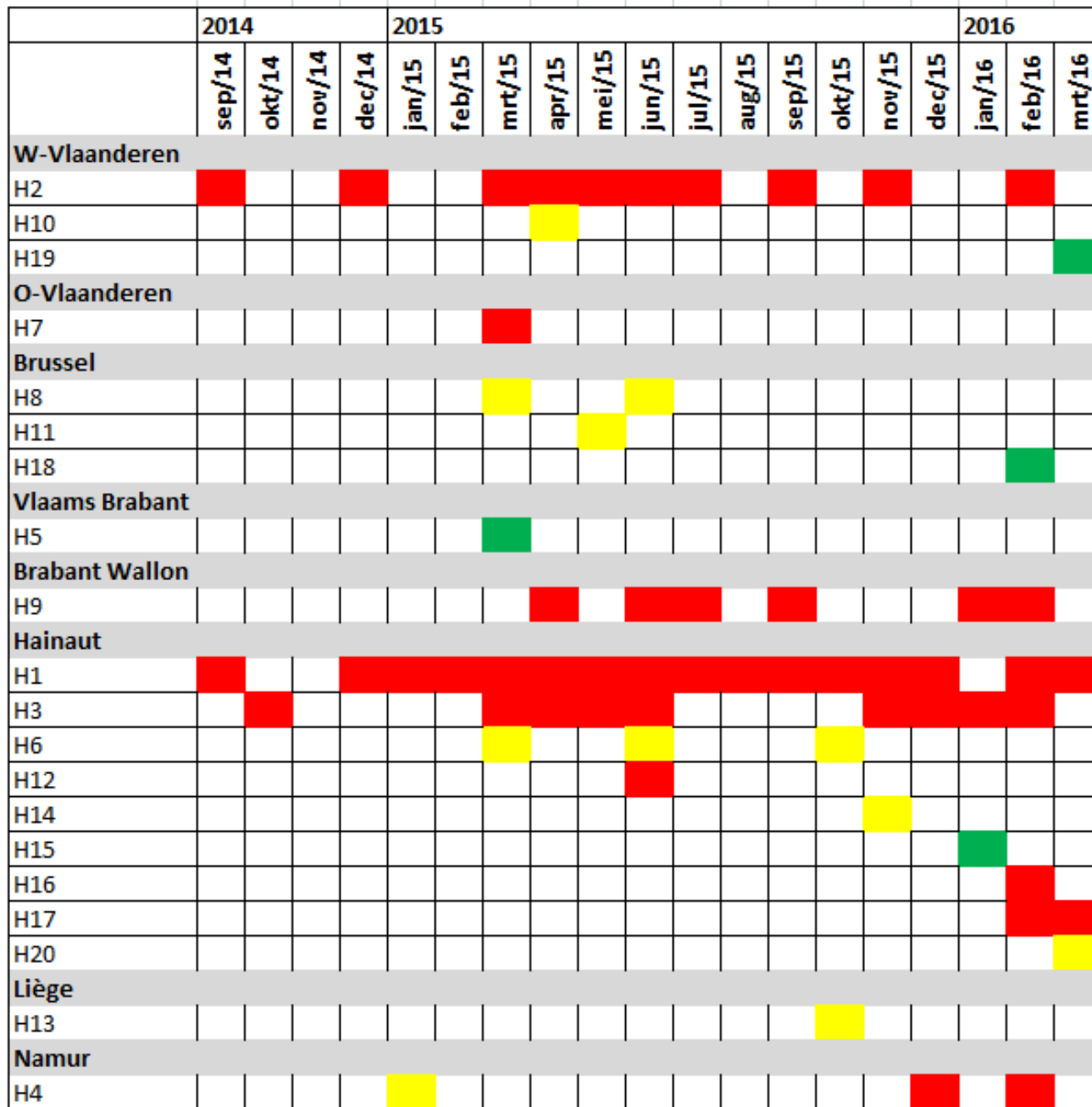


***Enterococcus faecium*,
vanA, PFGE-type 62 and 62-
like
(n=221 isolates)**

2016 (Q1)



Timeline: *E. faecium*, PFGE-type 62 and 62-like isolates



ISOLATES FROM:

- Outbreak
- No outbreak
- Unknown

VRE *E. faecium* outbreaks are increasing across Europe

- **Portugal:** increase among haemodialysis patients

Correia S. et al. 2014 Enferm Infecc Microbiol Clin.

- **Ireland:** rise from 33% in 2007 to 45% in 2012, mainly **ST17, ST18, ST78** and **ST203**

Ryan L et al. 2015. J. Antimicrob. Chemother.

- **Denmark:** increase since 2012

Pinholt et al. 2015. J. Antimicrob. Chemother.

- **Germany:** increase over of the years; most prevalent: **ST117, ST192, ST78, ST80**

Werner G. et al. 2016, ECCMID. P0658

- **The Netherlands:** increase since 2011, mainly **ST117, ST203, ST18**

Willems R, 2014, ECCMID O114

- **Norway and Poland:** mainly **ST117**

Werner G. et al. 2016, ECCMID. P0658

- **Italy:** decrease in bloodstream infections from 24.1% in 2003 to 4.5% in 2013, mainly ST78, ST202, **ST17**

Del Grosso et al. 2015, Antimicrob. Agents Chemother.

- **Spain:** spread of **ST117** across several regions

De Benito I. et al. 2013, ECCMID R2632;

Pedrosa-Tedim AS. et al. 2016, ECCMID, P0657

(Re-) Emerging strains?

- Isolation of rare Enterococcal species from clinical specimens:
 - *E. gilvus*, *E. mundtii*, *E. avium*, *E. durans*, *E. cecorum*
- *E. faecium* *vanA* + *vanB* positive:
 - N=4: $\frac{3}{4}$ same PFGE-type, same hospital
- *E. faecium*: 2 *vanD* positive
- *E. gallinarum*: *vanA*+ *vanC* positive (MIC vanco 8; MIC teico 128)
- *E. faecium*: tigecycline R (MIC=4.0),
 - *vanA*+,
 - linezolid S
- *E. faecalis*: tigecycline R (MIC=4.0), patient on omeprazole treatment (cfr Cordina et al. J. Antimicrob. Chemother. 2012, Werner et al. J. Antimicrob. Chemother. 2008)
 - vanco S,
 - linezolid S

Emerging strains? Linezolid R enterococci

- Linezolid R: 2 resistance mechanisms
 - Point mutations in 23S rDNA, MIC ↑ in function of copy number affected
Alonso et al. J. Microbiol. Meth. 2014
 - Presence of cfr-gene (high MIC)
Diaz et al. Antimicrob. Agents and Chemother. 2012

- Nr of linezolid R strains received : **2.3%**

2013 (done for 70/178 strains)

- *E. faecalis*: n=1 (1/1 vanco **S**) MIC 8.0
- *E. faecium*: n=2 (1/2 also vanco R) MIC >8.0, 64

2014

- *E. faecalis*: n=7 (7/7 vanco **S**) MIC 8.0
- *E. faecium*: n=7 (6/7 also vanco R) MIC 8.0

2015

- *E. faecalis*: n=0
- *E. faecium*: n=2 (2/2 also vanco R), MIC 8.0

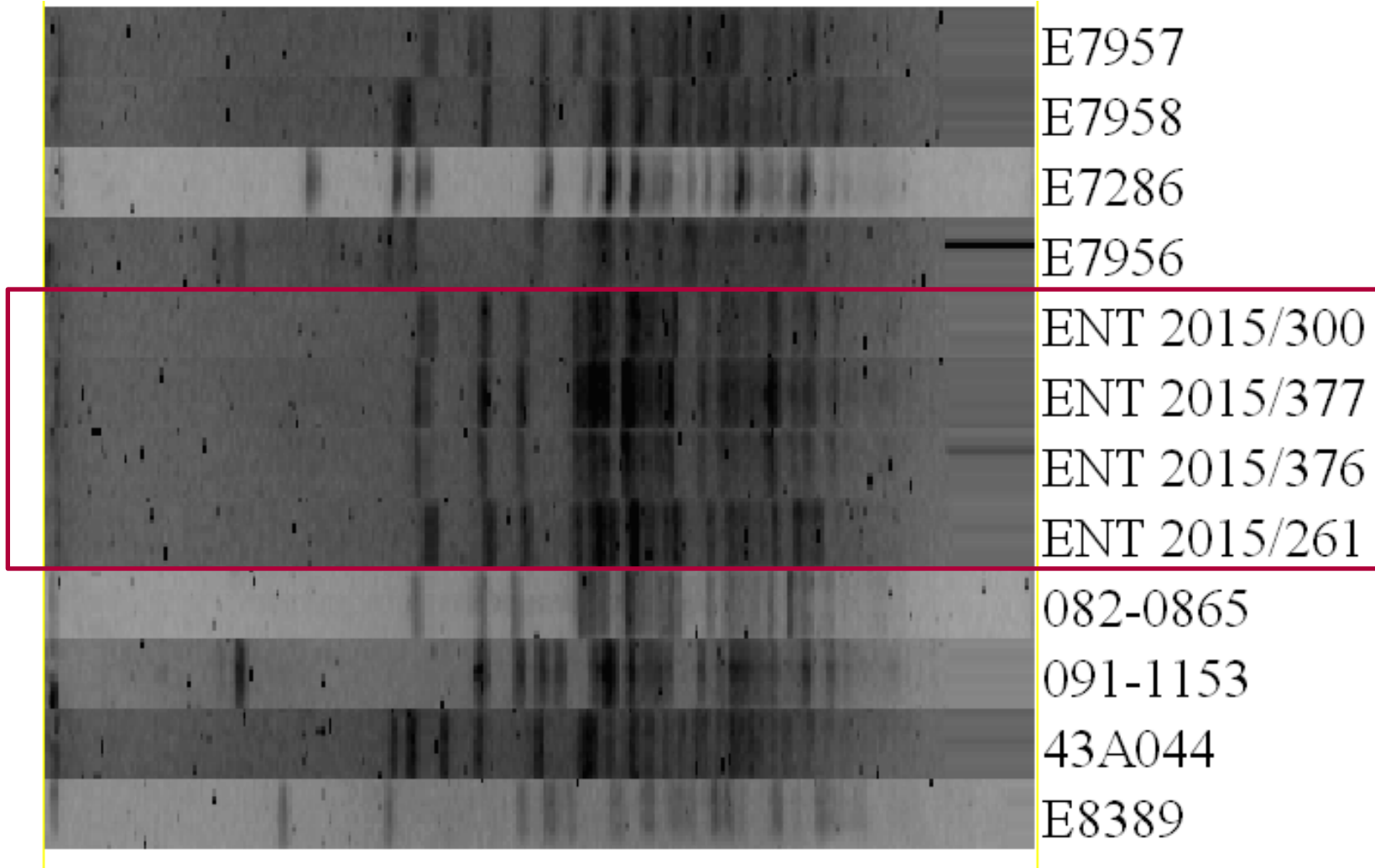
(Re-) Emerging strains? *E. raffinosus*

- Since 2006, The NRC received 2 strains of *E. raffinosus* of which 1 was vancomycin S, the other was *vanA* positive
 - 26/05/2015: receipt of a *E. raffinosus* strain at the NRC from hospital 1; *vanA* positive
 - 08/06/2015: receipt of a *E. raffinosus* strain at the NRC from hospital 2; *vanA* positive
 - Both isolates were detected by routine screening procedures
 - Both hospitals were from the same region
 - Both patients stayed at the same nursing home
 - An identical PFGE-type was obtained
 - After further screening by the Public Health Authorities, 2 more isolates were detected
 - ***vanA* positive *E. raffinosus* rarely identified in outbreaks**

Kawalec et al. Clin. Microbiol. Infect. 2007;

Correia et al. Enferm Infecc Microbiol Clin. 2014.

PFGE *E. raffinosus*



Emerging strains? VVE: vancomycin variable *Enterococcus faecium*

- **VVE: vancomycin variable *Enterococcus faecium***
 - Susceptible to vancomycin and teicoplanin but *vanA* positive
Gagnon et al. J Antimicrob Chemother. 2011
 - Major nucleotide deletions in Tn1546
 - Strains can become vancomycin R in the presence of vancomycin
Coburn et al. J. Clin. Microbiol. 2014;
Thaker et al. J Antimicrob Chemother. 2015
 - Outbreaks have been described in Canada, Norway
Szakacs et al. J. Clin. Microbiol. 2014;
Sivertsen et al. 2015, ECCMID 2015, EV01721
 - Spring 2015: first 2 VVE strains received by the NRC
 - Case 1: 04/04/2015, urinary tract infection, local MIC vancomycin 8 and teicoplanin 1 by Vitek2, but MIC vancomycin 2, teicoplanin 3 by e-test)
 - Case 2: 30/03/2015, sepsis in oncology patient, treated with vancomycin without success.
 - Characterisation of the *vanA* operon confirmed the presence of VVE!

Conclusions and remarks

- Increasing number of enterococcal strains submitted to the NRC
- So far no legal obligation for laboratories to submit their strains. Difficult to conclude on the observed trends.
- By applying more sophisticated detection techniques rare enterococcal species are also isolated from human specimens and submitted for confirmation to the NRC.
- Most prevalent type of acquired VAN-resistance is still *vanA*
- ST80, 117 and 203 are the most prevalent *E. faecium* types in Belgium (conform the Netherlands, Sweden)
- Increasing number of outbreaks by *E. faecium* (conform the situation in the Netherlands, Sweden), each hospital has more or less his own “pet”.
- (Re-) emerging strains: VVE, tigecycline resistant strains, linezolid resistant strains, ... ⇒ should be submitted to the NRC for confirmation and epidemiological surveillance across Belgium