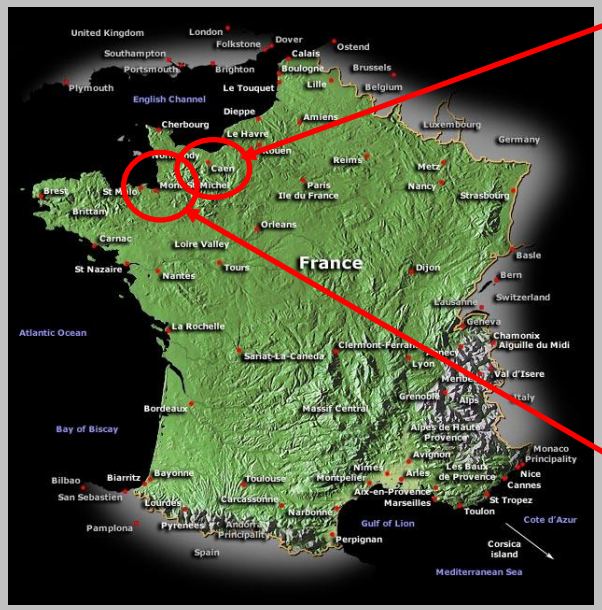
Current issues with VRE outbreaks and experience with rapid detection using the Xpert™ VanA/VanB assay

Roland Leclercq, University Hospital of Caen, France
National Reference Center for antimicrobial resistance
(associated laboratory for enterococci)

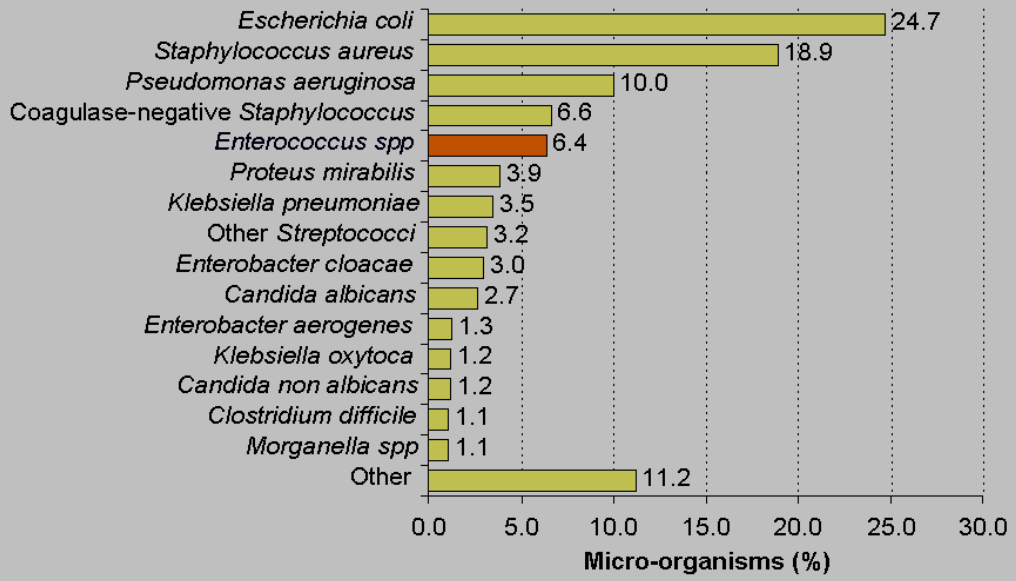


University Hospital of Caen (Normandy, France), 1700 beds.

8th Century Abbey Mount Saint Michel

Enterococci at the hospital

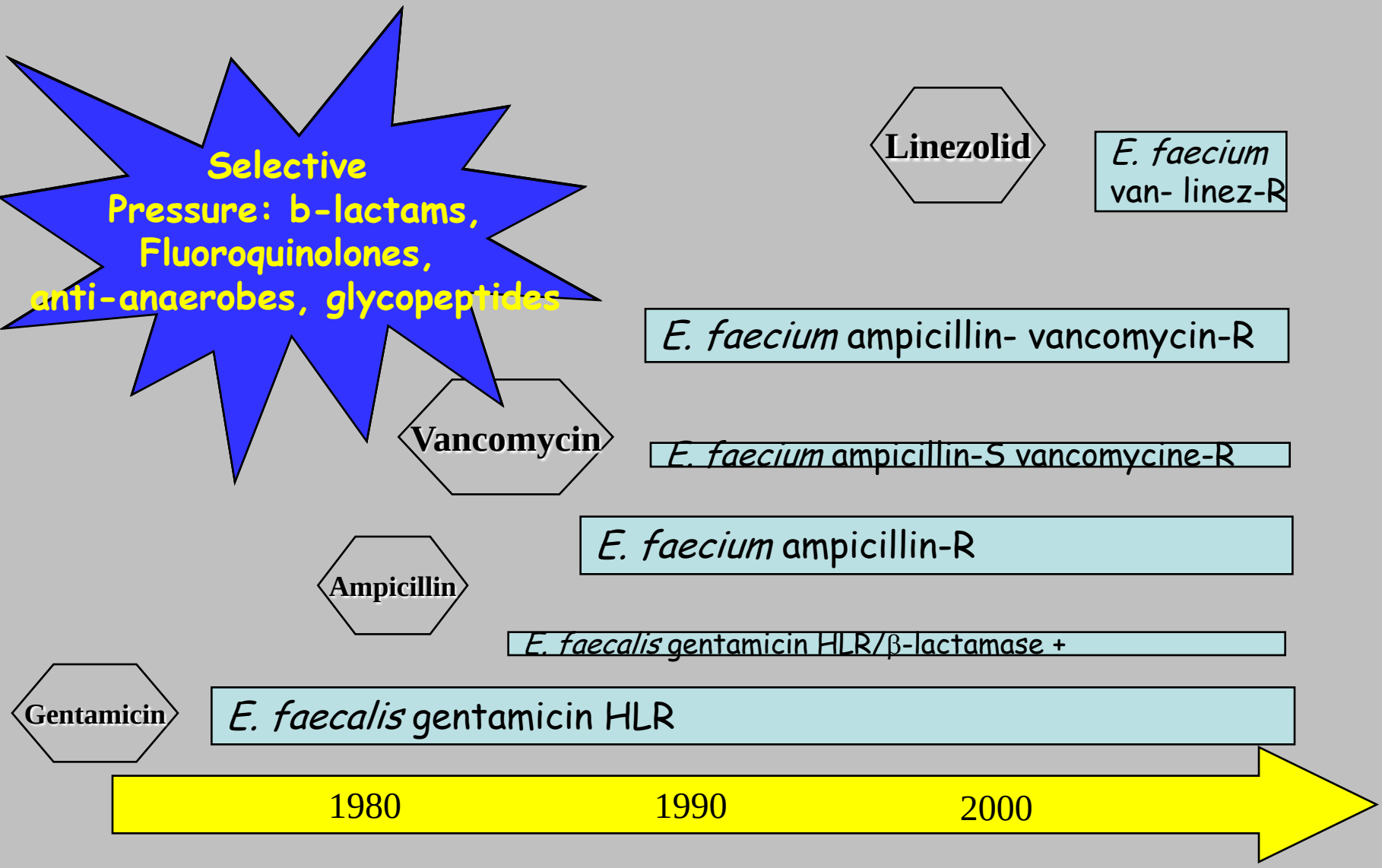
- A major nosocomial pathogen in many countries
  - 3rd to 6th most prevalent genus in hospital-acquired infections



Source: 2006 French PPS
Raisin (http://www.invs.sante.fr/raisin/)

- *E. faecalis* (85-90%) and *E. faecium* (10%) infections. However, proportion of *E. faecium* is increasing (up to 35%).

Multiple antibiotic resistant enterococci

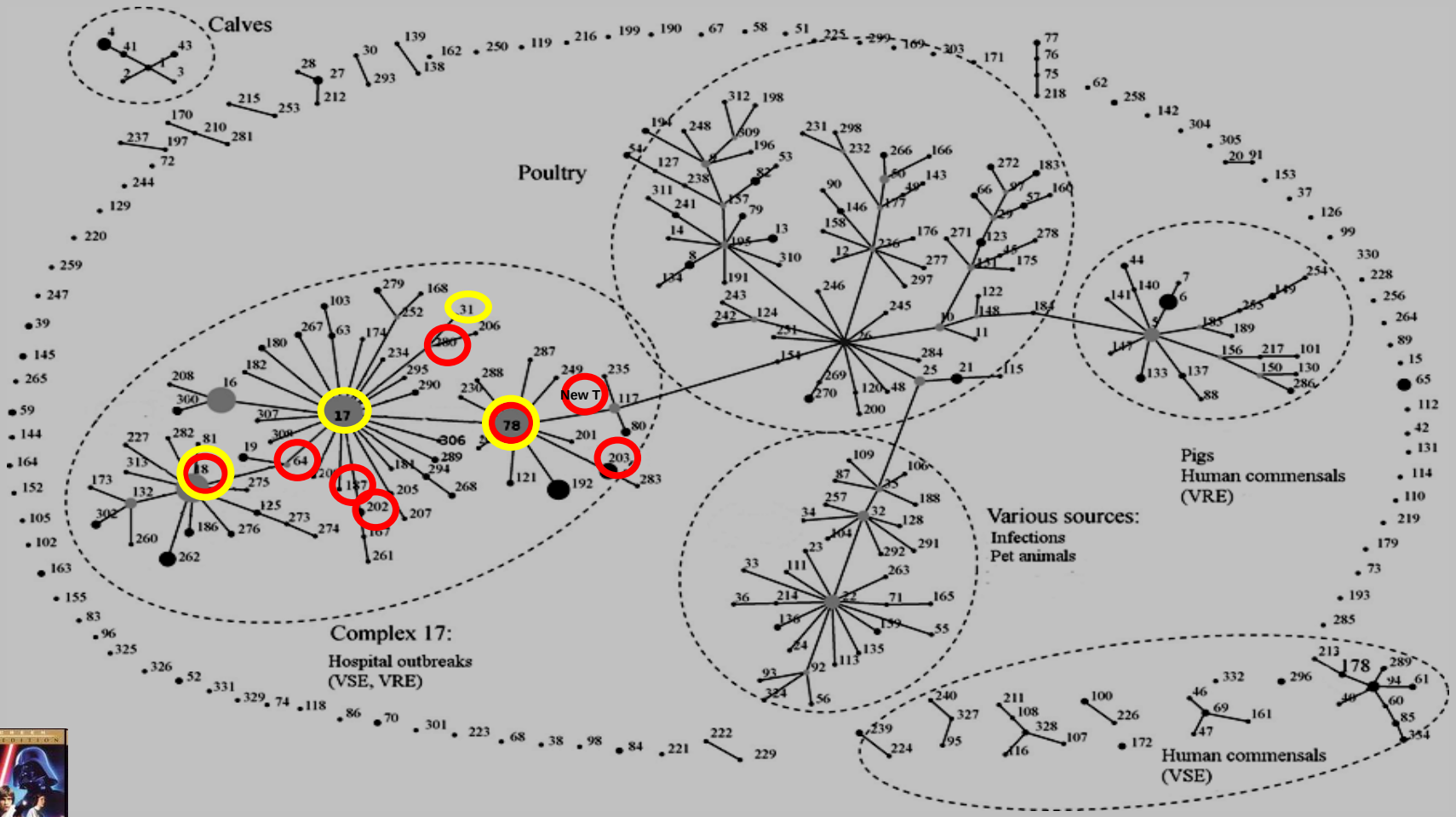


Particular enterococci

- MultiLocus Sequence Typing (MLST) of *E. faecium* isolates revealed the existence of host-specific genogroups, including a specific clonal complex designated CC17, associated with hospital-related isolates.
- CC17 isolates are
  - Resistant to ampicillin and quinolones.
  - Most contain particular genes: mobile elements, phage genes, genes encoding membrane proteins, regulatory genes, a putative pathogenicity island including the *esp* gene, and megaplasmids.

Leavis HL et al. Emerg Infect Dis. 2003;9:1108-15.
Klare I, et al. Eur J Clin Microbiol Infect Dis. 2005:815-25.
Top J, Willems R, Bonten M. FEMS Immunol Med Microbiol. 2008;52:297-308
Freitas AR et al. Antimicrob Agents Chemother. 2010;54:2660-5.

«Star wars: Attack of the clones»



Top J, Willems R, Bonten M. FEMS Immunol Med Microbiol. 2008;52:297-308

E-burst

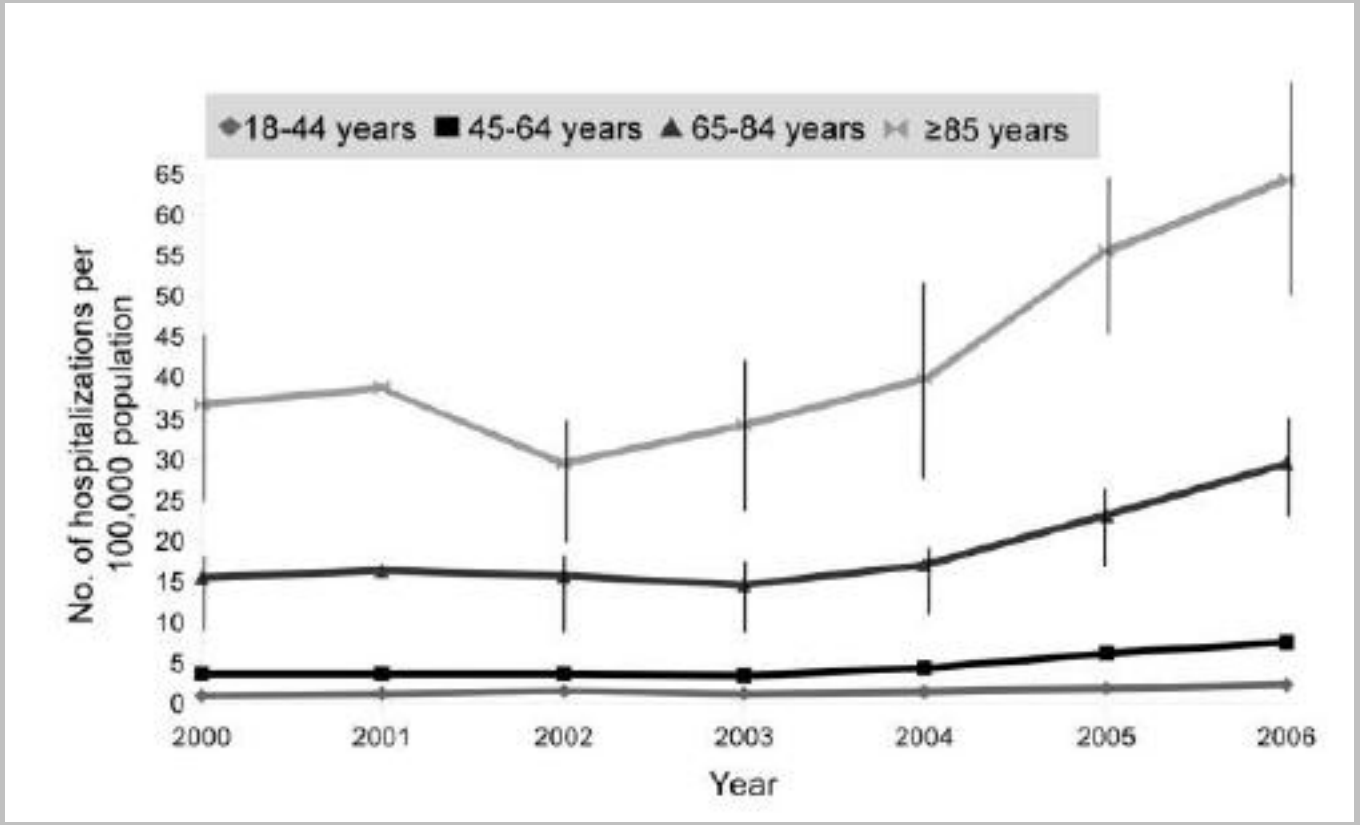
Worldwide dissemination of VRE

- Initially VRE were reported in France and the United Kingdom in 1987, and then in the rest of Europe and in the USA

- Since 1995, they are reported worldwide.

  E. faecium with the VanA-type (cross resistance vanco/teico) or the VanB-type (resistance to vancomycin only) are widely predominant

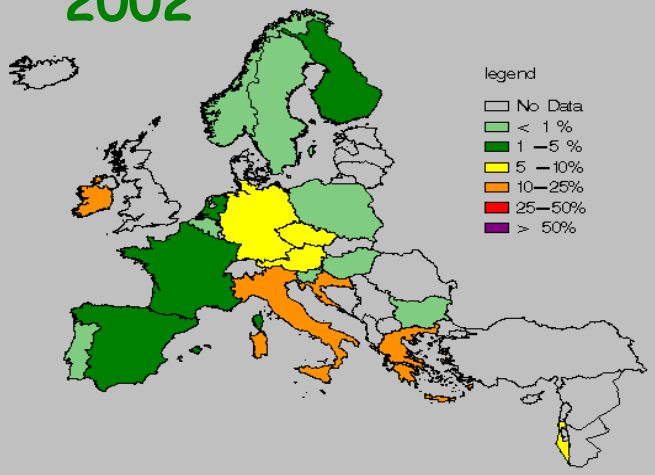
Age-specific increase in hospitalizations due to VRE infections (USA 2000-2006)



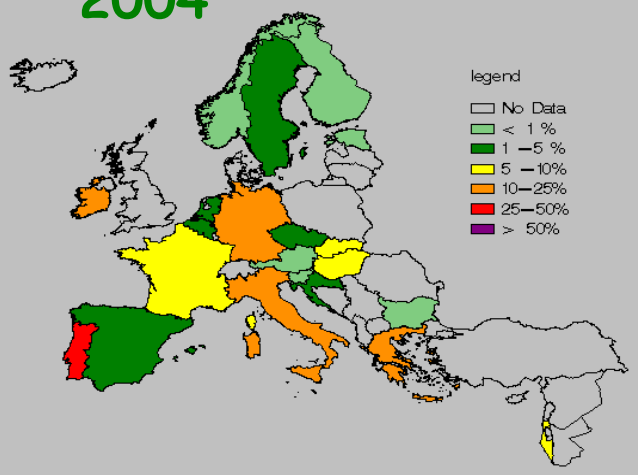
Ramsey AM et al. ICHE. 2009: 30; 184-187

E. faecium in Europe: % VR *E. faecium* in blood cultures (EARSS http://www.earss.rivm.nl/)

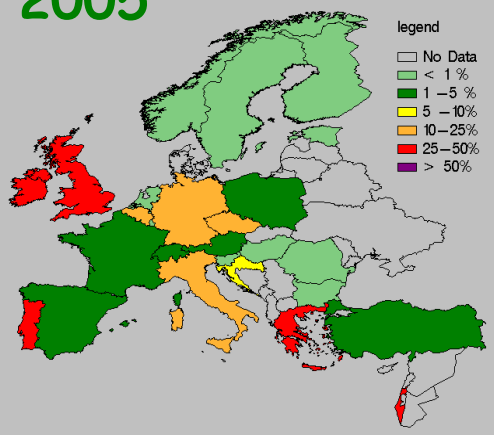
2002



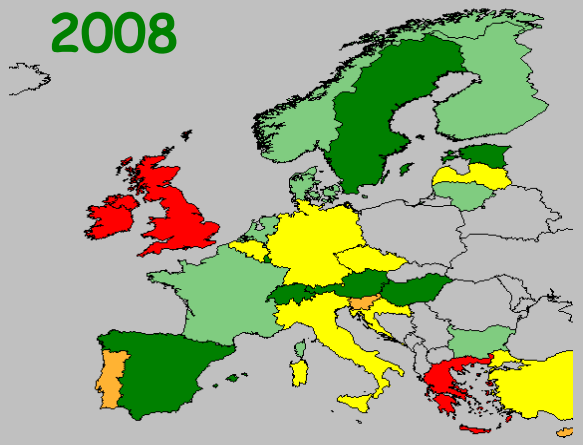
2004



2005



2008



Skin and soft tissue infections (Europe)

Country	% MRSA (no. of *S. aureus* tested)	% VRE (no. of enterococci tested)
Belgium	48.4 (31)	0.0 (9)
France	25.0 (517)	0.0 (51)
Germany	13.7 (365)	2.7 (75)
Greece	42.5 (80)	35.3 (17)
Ireland	43.3 (134)	9.5 (21)
Israel	26.8 (87)	0.0 (13)
Italy	27.4 (197)	2.6 (38)
Poland	33.3 (72)	63.6 (11)
Russia	3.0 (34)	– (0)
Spain	21.6 (213)	0.0 (21)
Sweden	0.4 (236)	0.0 (40)
Switzerland	15.4 (91)	0.0 (14)
Turkey	11.7 (128)	15.8 (19)
UK	27.5 (356)	25.0 (4)
Overall	22.5 (2541)	5.1 (333)

Enterococci isolated in 9.3% of samples

Sader HS et al. Int J Antimicrob Agents. 2010; 36:28-32

VRE 2007-08

Enterococcus	% of resistance to vancomycin according to region (no of isolates)				
	APAC	Europe	Latin America	North America	Overall
faecium	14.1 (270)	31.5 (489)	48.1 (54)	76 (597)	47.6 (1410)
faecalis	0.01 (440)	1.5 (919)	3 (195)	5.6 (945)	3 (2499)
All	11.9 (710)	11.9 (1408)	12.9 (249)	32.8 (1542)	19.1 (3909)

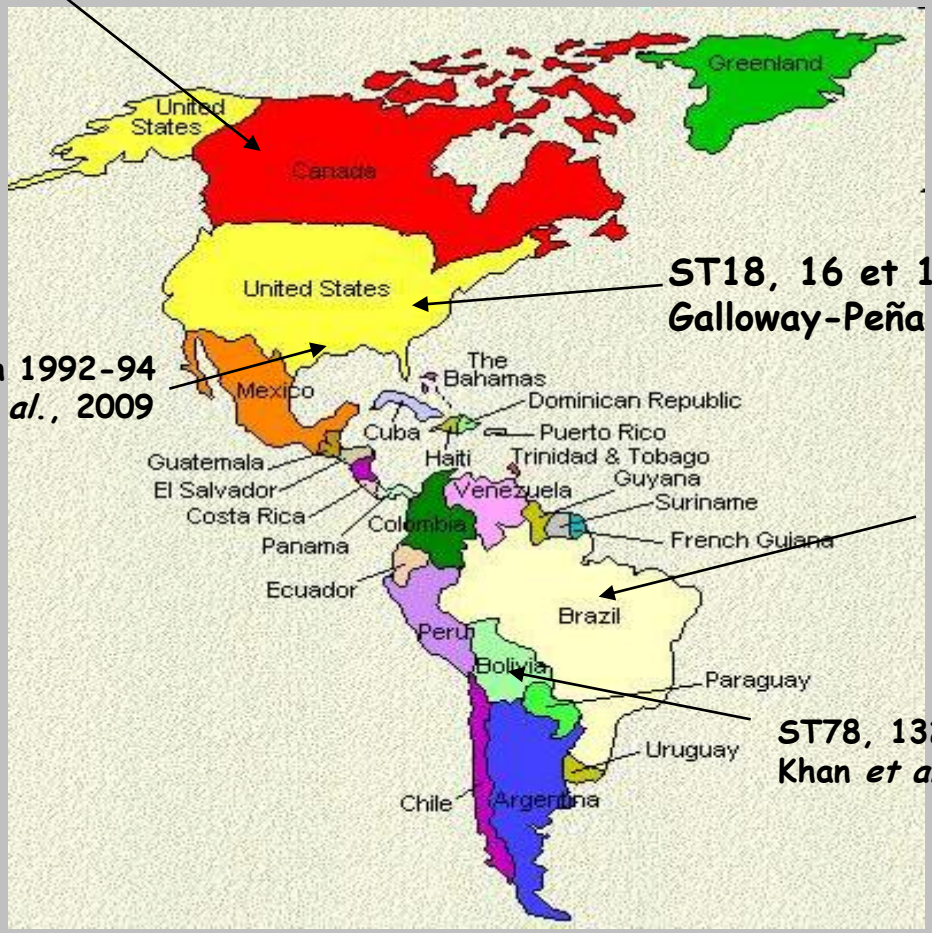
Enterococcus spp. were from blood (58.3%), urine (14.1%), and wounds (7.7%)

E. faecium: 2/3 from blood, 8% from urine.

Putnam SD et al. Diagn. Microbiol. Infect. Dis. 2010; 67:359-68

America

VRE low prevalence, mostly *vanA*
Zhanel *et al.* et Ofner-Agostini *et al.*, 2008



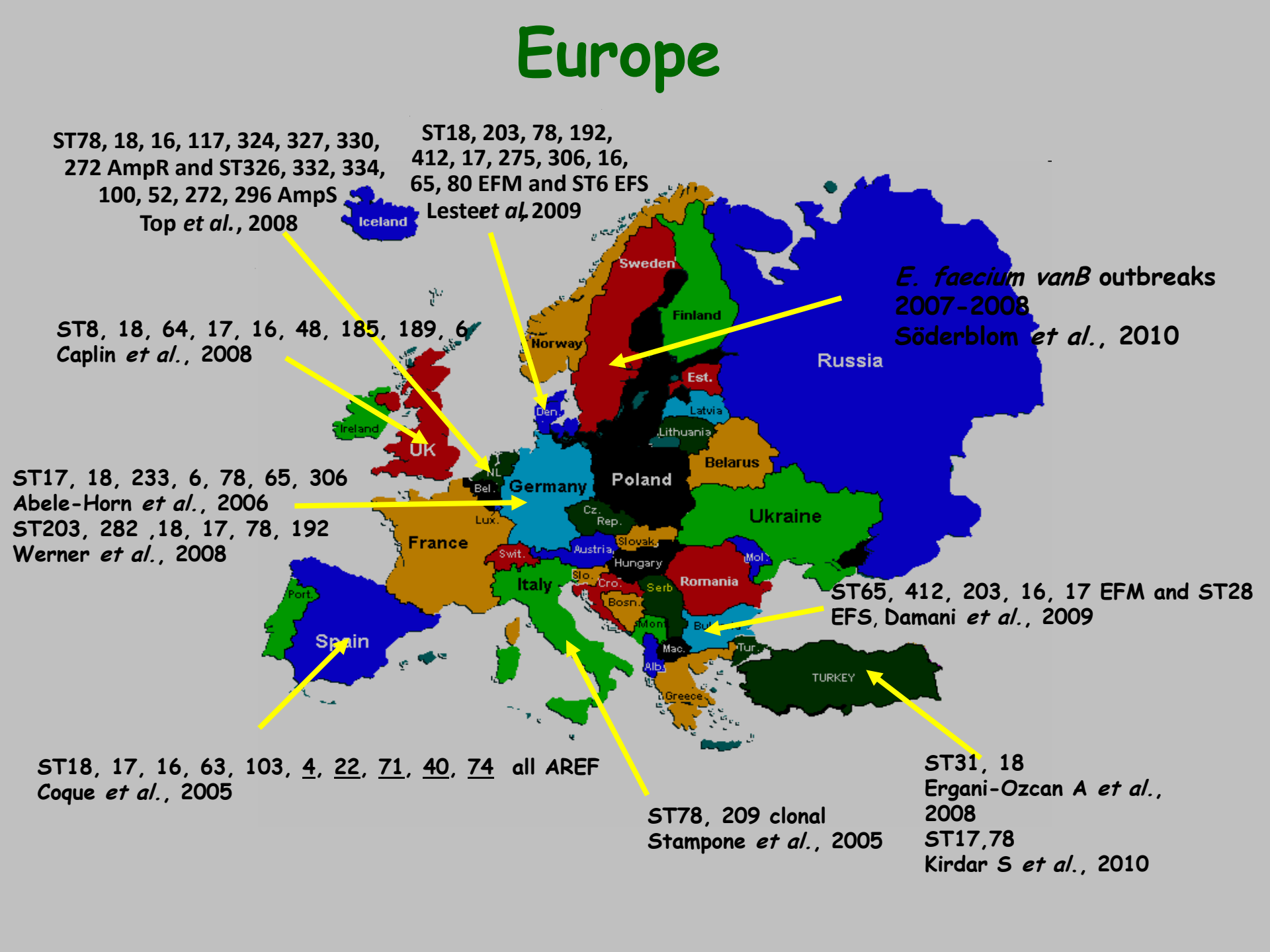
ST18, 16 et 17 in 1990-91
Galloway-Peña *et al.*, 2009

ST20, 17, 475 in 1992-94
Galloway-Peña *et al.*, 2009

ST114, 17, 281 et 50
Camargo *et al.*, 2008

ST78, 132, 210 and 438 all *vanA* and 97% *esp+*
Khan *et al.*, 2009

Europe


ST78, 18, 16, 117, 324, 327, 330, 272 AmpR and ST326, 332, 334, 100, 52, 272, 296 AmpS
Top *et al.*, 2008
ST18, 203, 78, 192, 412, 17, 275, 306, 16, 65, 80 EFM and ST6 EFS
Leste*et al*, 2009
E. faecium vanB outbreaks 2007-2008
Söderblom *et al.*, 2010
ST8, 18, 64, 17, 16, 48, 185, 189, 6
Caplin *et al.*, 2008
ST17, 18, 233, 6, 78, 65, 306
Abele-Horn *et al.*, 2006
ST203, 282 ,18, 17, 78, 192
Werner *et al.*, 2008
ST65, 412, 203, 16, 17 EFM and ST28 EFS, Damani *et al.*, 2009
ST18, 17, 16, 63, 103, 4, 22, 71, 40, 74 all AREF
Coque *et al.*, 2005
ST78, 209 clonal
Stampone *et al.*, 2005
ST31, 18
Ergani-Ozcan A *et al.*, 2008
ST17,78
Kirdar S *et al.*, 2010
Iceland
Sweden
Finland
Norway
Russia
Est.
Latvia
Lithuania
Den.
Ireland
UK
NL
Bel.
Germany
Poland
Belarus
Ukraine
Cz. Rep.
Lux.
France
Slovak.
Austria
Swit.
Hungary
Mol.
Slo.
Cro.
Serb
Romania
Italy
Bosn.
Port.
Spain
Mont
Bul
Mac.
Tur.
Alb.
TURKEY
Greece

Australia and New-Zealand

E. faecalis vanA
Manson *et al.*, 2004

First VRE 1994
E. faecium vanB > *vanA* > *E. faecalis vanB* > *vanA*
Bell *et al.*, 1998, Christiansen *et al.*, 2004 et 2007, Worth *et al.*, 2008

Asia

ST18, 25, 78, 203, 11, 280, 320, 321, 322, 323, 335
Zheng *et al.*, 2007
ST78, 117, 203, 316, 362, 363, 364, 365
Zhu *et al.*, 2009

ST78, 192, 203, 17, 204 ,205, 206, 207
Soo Ko *et al.*, 2005

EFM vanA
Yaslani *et al.*, 2009

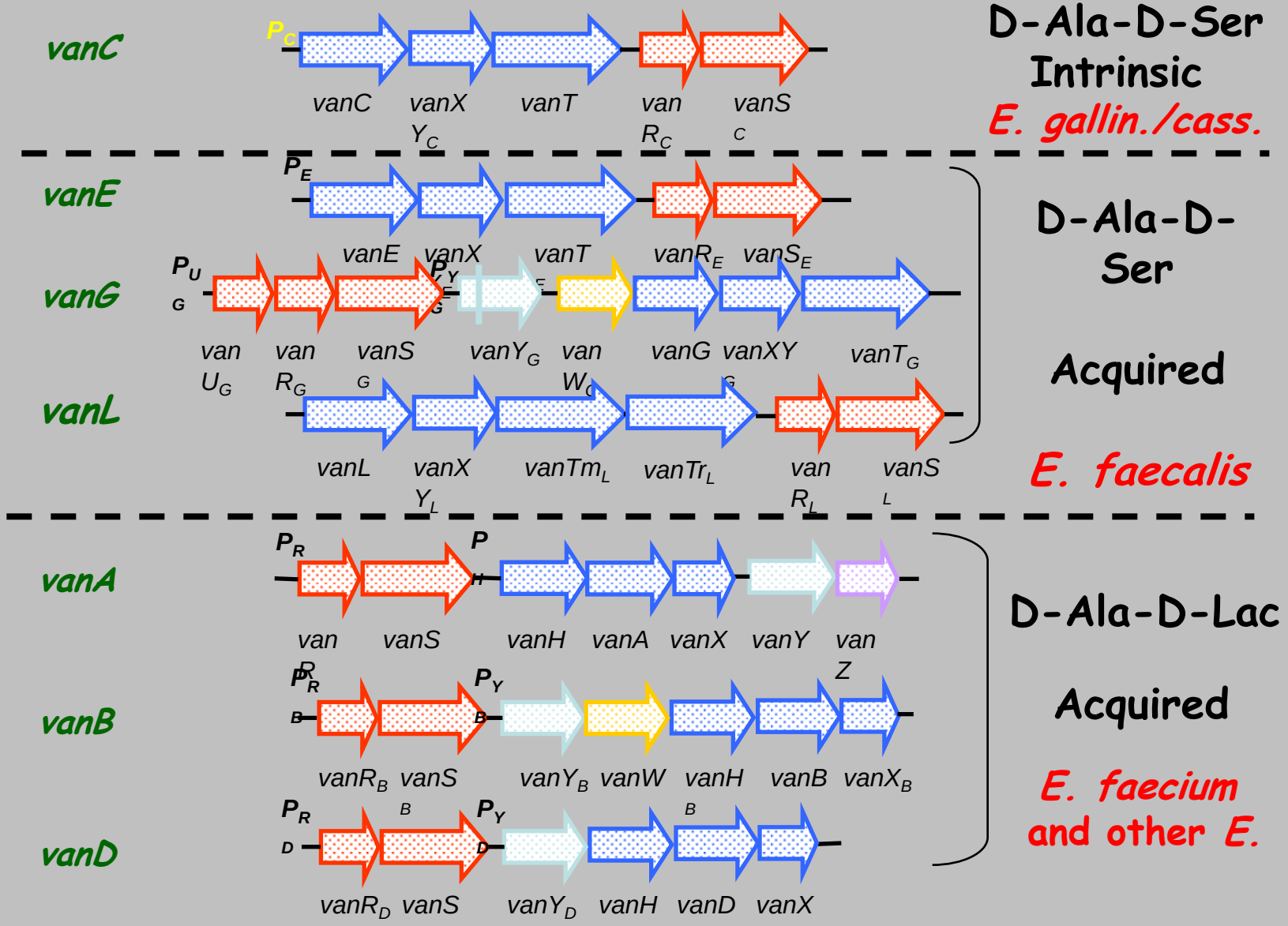
EFM vanA
De *et al.*, 2009

EFM>EFS vanA
Koh *et al.*, 2009

First VRE isolated in 1996
ST78, 359, 343, 18, 444
Hsieh *et al.*, 2009

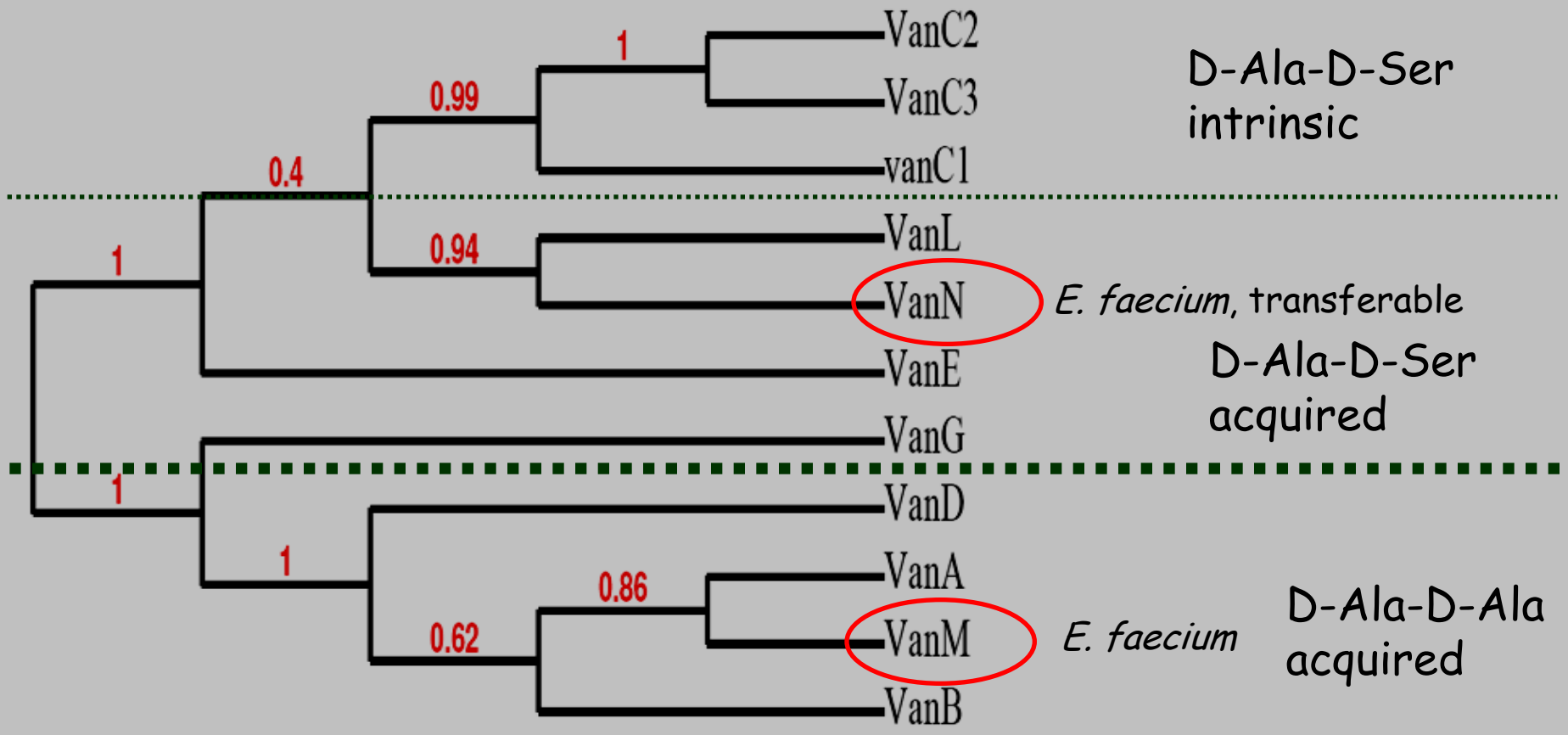
A diversity of resistance genes

The seven *van* operons



F. Depardieu, I. Podglajen, R. Leclercq, E. Collatz, P. Courvalin. Clin Microbiol Rev 2007

Two more in the *van* alphabet



Xu X, et al. Antimicrob Agents Chemother. 2010 Aug 23.
Lebreton F et al. ESCMID, Vienna 2010

A huge reservoir

- Unsuspected carrier patients are a major reservoir
  - For one patient found positive in a clinical sample, between 2 and 10 contact patients are carriers
- Reservoir of vancomycin resistance genes not limited to enterococci and not limited to humans

The anaerobes as reservoir of *van* genes

- *van* genes (*vanA*, *vanB*) have been detected in other species than enterococci

- The *vanB* genes and Tn*1549*-like element have been detected in *Clostridium* sp., *Eggerthella lenta, and Ruminococcus* sp. Also, *vanD* and *vanG* in *Ruminococcus*

- *Clostridium symbiosum* MLG101 transferred its Tn1549-like element (*vanB*) to *E. faecium* and *E. faecalis* in the digestive tract of gnotobiotic mice

Stinear TP et al. The Lancet 2001; 357:855-6
Ballard SA, et al. Antimicrob Agents Chemother. 2005;49:1688-94.
Launay A et al. Antimicrob Agents Chemother. 2006;50:1054-62.
Domingo et al.,. Antimicrob. Agents Chemother., 2007

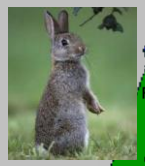
The animal reservoir

- Ducks, chicken, pigs, horses, cows, goats, pets are carriers
- Urban and hospital wastewaters
- Various food products, meat, vegetables, cheese
- Contamination of meat by houseflies (generally $8x10^4$ cfu of enterococci within 30 minutes) (Macovei et al. J Food Protect, 2008;71:435-9).

Heterogeneity of strains and frequent presence of Tn*1546* (*vanA*)
→ Spread of genes rather than spread of strains

Into the wild

CC17



Badgers, wild boars, wild rabbits, woodmices, polar gulls

Mallon DJ, et al. Emerg Infect Dis. 2002;8:636-8.

Poeta P, et al. Vet Microbiol. 2007;125:368-74.

Drobni M et al. Emerg Infect Dis. 2009;15:838-9.

Silva N, et al . Sci Total Environ. 2010;408:4871-6

Who is afraid of VRE?

Multidrug resistance

Pathogenicity

Transfer of vancomycin resistance to MRSA

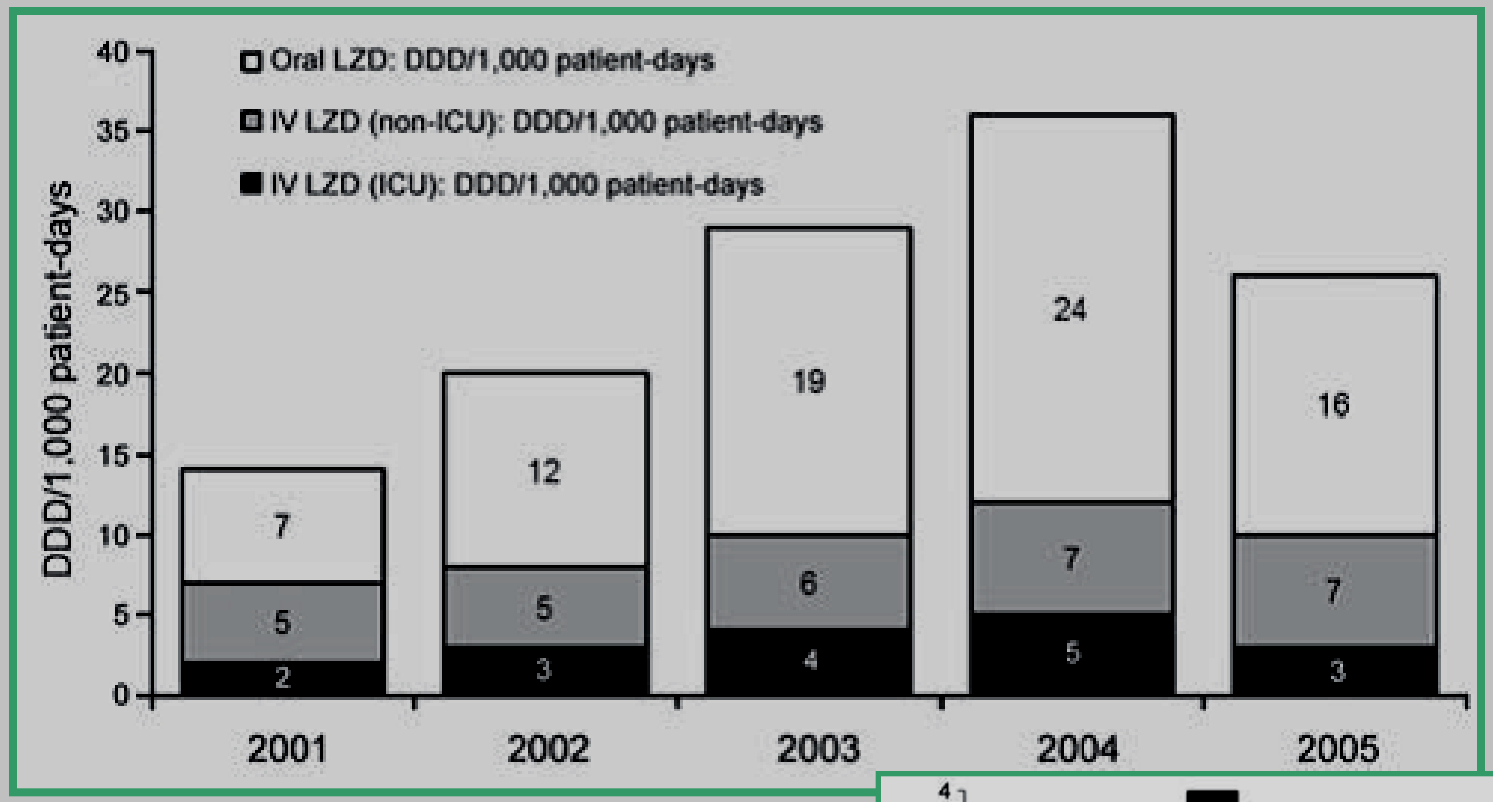
Multiple antibiotic resistance

TABLE 1. Proportion (%) of VRE clinical isolates resistant to antibiotics (other than glycopeptides) according to species and genotype.

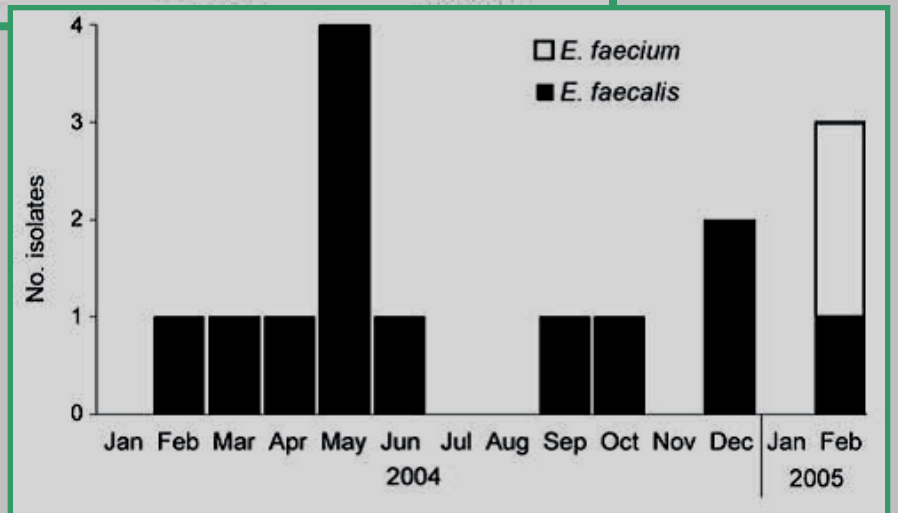
Antibiotic	Proportion (%) resistance					
	E. faecium (n = 604)			*E. faecalis* (n = 30)		Other
	VanA (n = 441)	VanB (n = 161)	VanD (n = 2)	VanA (n = 23)	VanB (n = 7)	species[a] (n = 15)
Ampicillin	93.7	100	100	0	0	13.3
Streptomycin	51.5	77.4	-[b]	53.8	57.1	13.3
Kanamycin	78.5	99.4	100	69.6	85.7	33.3
Gentamicin	21.8	23.6	50	60.9	57.1	13.3
Chloramphenicol	2.5	1.2	0	30.4	28.6	20.0
Doxycyclin	63	3.7	100	87	85.7	73.3
Tigecyclin	0	0	0	4.3	0	0
Erythromycin	99.1	100	100	95.7	71.4	46.6
Lincomycin	95.5	95.7	100	100	100	100
Pristinamycin	0.7	0	0	100	71.4	0
Levofloxacin	91.8	97.5	50	69.6	42.9	6.6
Linezolid	0	0	0	0	0	0
TMP-SMX[c]	67.6	90.7	0	60.9	42.9	6.6
Rifampicin	8.8	1.9	0	0	0	6.6
Fusidic Acid	0.5	0	0	0	0	0

Daptomycin active in vitro: not licensed, rare resistant isolates, suboptimal dosages?
Quinupristin-dalfopristin: resistance

Outbreaks of linezolid-resistant enterococci



- Mostly G2576 mutation in clinical isolates
- Mutations of ribosomal proteins L3 and L4

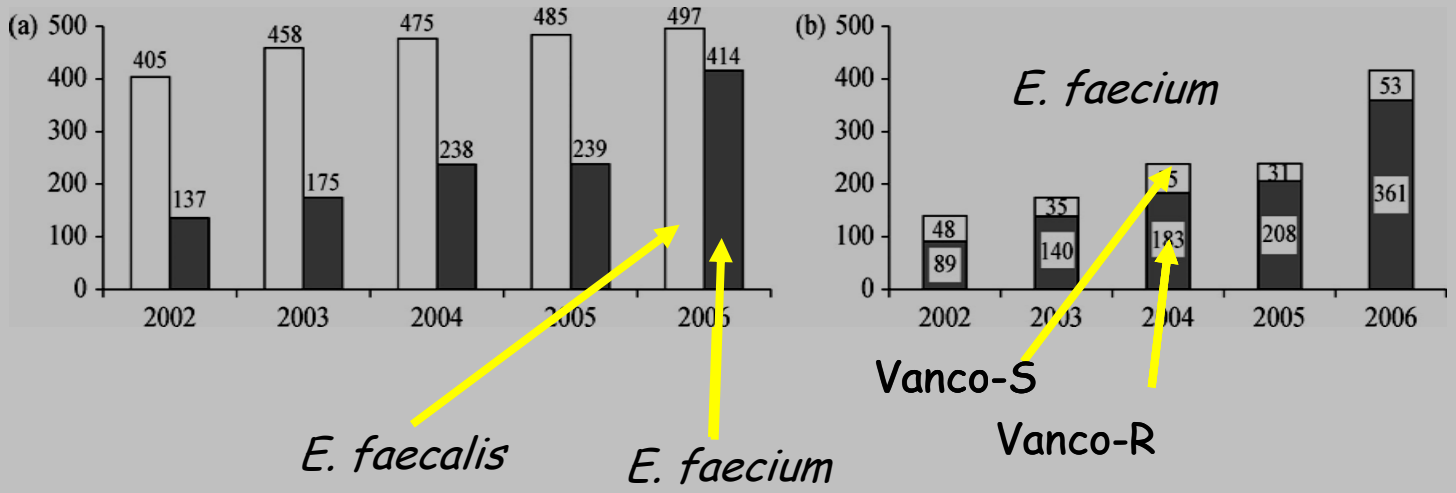


Kainer et al., Emerg Infect Dis, 2007;13: 1024-1030

VRE: a feeble pathogen?

-VRE are considered feeble pathogens (Ratio infections/colonisations 5-10%)
- Increase in infections?

Enterococcal blood culture isolates from 11 Danish counties (2002 to 2006)



Lester CH, et al. J Antimicrob Chemother. 2008; 62:1203-6.

Vancomycin-R MRSA: Apocalypse now?

- Despite initial fears, only few *S. aureus* acquired *vanA* and did not spread
  - Nine isolates in the USA (7/9 in Michigan) and two other reports (Iran, India).
  - No spread although the US isolates belong to ST5 (includes USA100, USA800)

Tenover F. Clin Infect Dis. 2008; 46:675-6
Sievert MS et al. Clin. Infect. Dis. 2008; 46:675-7
Emaneini M et al. J Hosp Infect. 2007;66:92-3
Saha B, et al. J Med Microbiol. 2008;57:72-9.
Finks J et al. Emerg Infect Dis. 2009;15:943-6

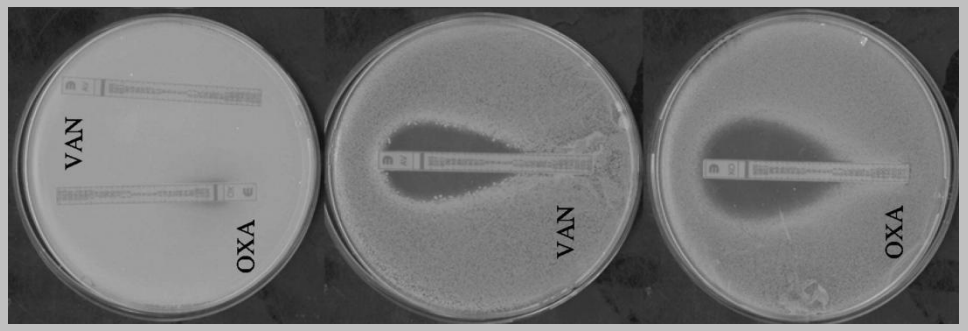
Lost in America?

- Plasmid-instability

- Low frequency of transfer (plasmid Inc18)

- Staphylococcal restriction enzymes are barriers for acquisition of vancomycin-resistance. Only few isolates are deficient in this system and easily acquire foreign genes

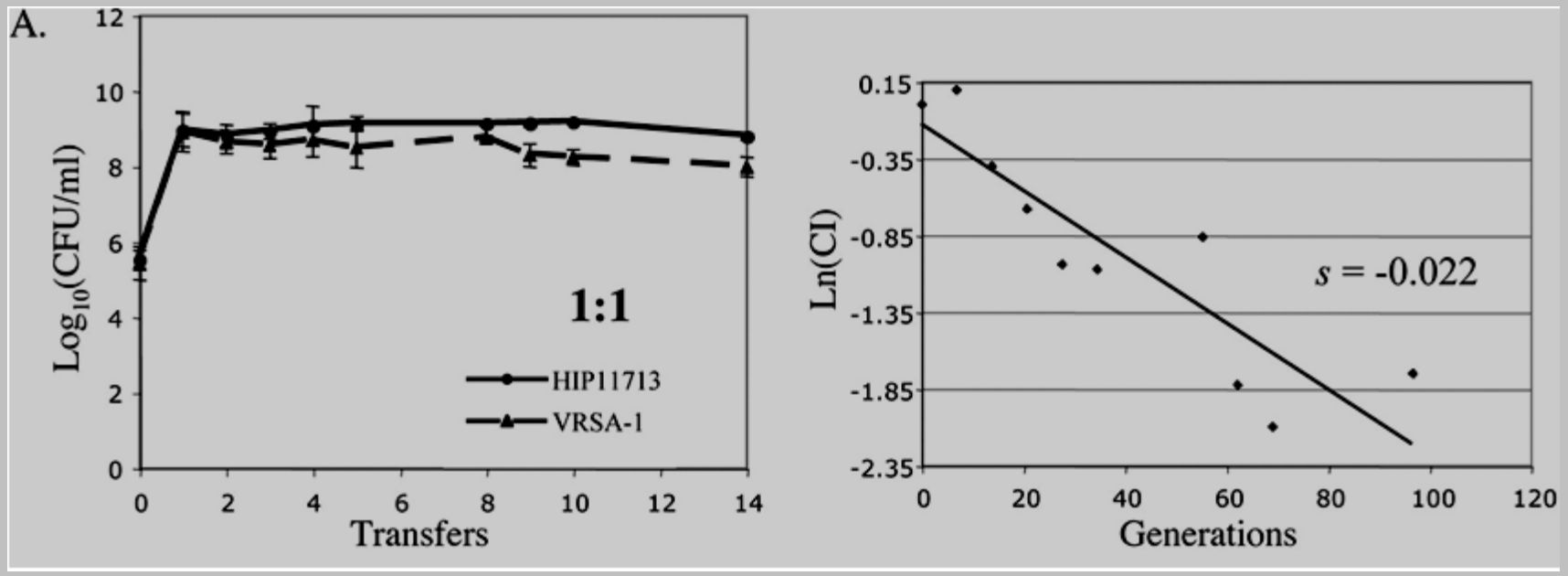
-Synergism between vancomycin and β-lactams (widely used in combination in ICUs)



Corvaglia AR, et al. Proc Natl Acad Sci U S A. 2010;107:11954-8.
Tenover F. Clin Infect Dis. 2008; 46:675-6
Périchon B, Courvalin P. Antimicrob Agents Chemother. 2006;50:3622-30.

Biological cost for *vanA* resistance in *S aureus*, but not in enterococci

Growth competition between VRSA-1 and HIP11713 mixed at an initial ratio of 1:1



In enterococci and in the absence of induction by vancomycin, tight regulation of resistance expression (VanRBSB two component system) drastically reduces the biological cost associated with Vm resistance in enterococci, favoring their dissemination.

Foucault, M.-L. et al. PNAS in Press

Foucault, M.-L. et al. 2009. Antimicrob. Agents Chemother. 53(6):2354-2359

Should we control VRE spread?

- Difficult to control (many unsuspected gut colonizations; huge gene reservoir)
- Few infections (<<10%)
- Other priorities…

- However
  - The number of infections will increase in the absence of control
  - Transfer of vancomycin resistance to staphylococci cannot be discarded

VRE Control works!

CONTROL OF VANCOMYCIN-RESISTANT ENTEROCOCCUS IN HEALTH CARE FACILITIES IN A REGION

BELINDA E. OSTROWSKY, M.D., M.P.H., WILLIAM E. TRICK, M.D., ANNETTE H. SOHN, M.D., STEPHEN B. QUIRK, M.P.P., STACEY HOLT, M.M.Sc., LORETTA A. CARSON, M.S., BERTHA C. HILL, B.S., MATTHEW J. ARDUINO, PH.D., MATTHEW J. KUEHNERT, M.D., AND WILLIAM R. JARVIS, M.D.

N Engl J Med, Vol. 344, No. 19 · May 10, 2001

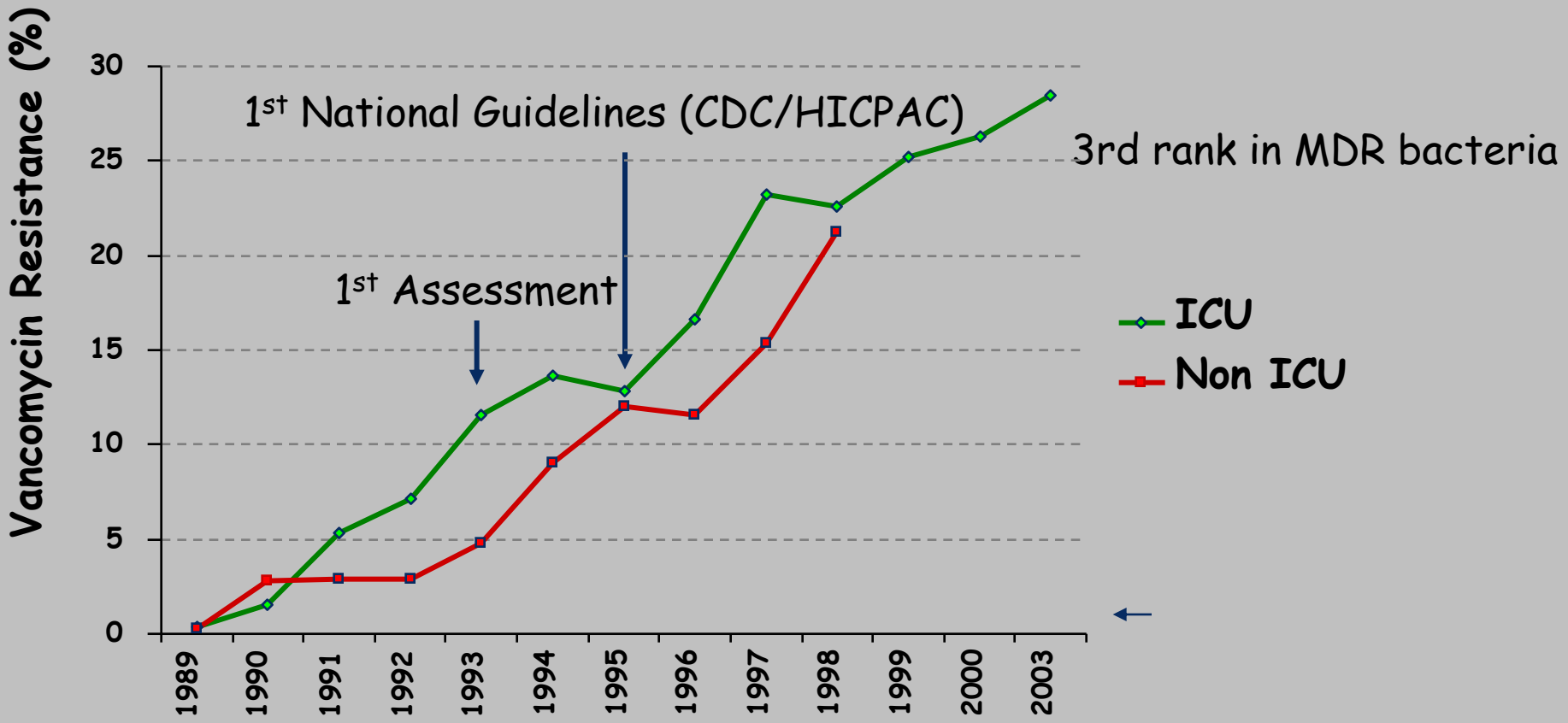
TABLE 3. PREVALENCE OF COLONIZATION WITH VANCOMYCIN-RESISTANT ENTEROCOCCI AMONG PATIENTS OR RESIDENTS OF 30 ACUTE CARE AND LONG-TERM CARE FACILITIES IN THE SIOUXLAND REGION IN JULY AND AUGUST 1997, OCTOBER 1998, AND OCTOBER 1999.*

TYPE OF FACILITY	COLONIZATION WITH VRE			1998 VERSUS 1997		1999 VERSUS 1998		1999 VERSUS 1997†	
	1997	1998	1999	RELATIVE RISK (95% CI)	P VALUE	RELATIVE RISK (95% CI)	P VALUE	RELATIVE RISK (95% CI)	P VALUE
	no. of patients (%)								
All	40 (2.2)	26 (1.4)	9 (0.5)	0.6 (0.4–1.1)	0.08	0.4 (0.2–0.8)	0.005	0.2 (0.1–0.5)	<0.001
Acute care	10 (6.6)	9 (5.5)	0	0.8 (0.4–2.0)	0.67	0	0.002	0	<0.001
Long-term care	30 (1.7)	17 (1.0)	9 (0.5)	0.6 (0.3–1.0)	0.05	0.6 (0.2–1.3)	0.14	0.3 (0.2–0.7)	0.001

*Only data from the 30 facilities that participated in all three years of the study were included. VRE denotes vancomycin-resistant enterococci, and CI confidence interval.

†The results of the chi-square test for trend for the overall rates for 1997, 1998, and 1999 were also significant (P<0.001).

Glycopeptide-Resistant *Enterococci*, USA, 1989 - 2003



Source: NNIS System, CDC

Failure of recommendations

HIS INTERNATIONAL CONFERENCE, EDINBURGH 2002

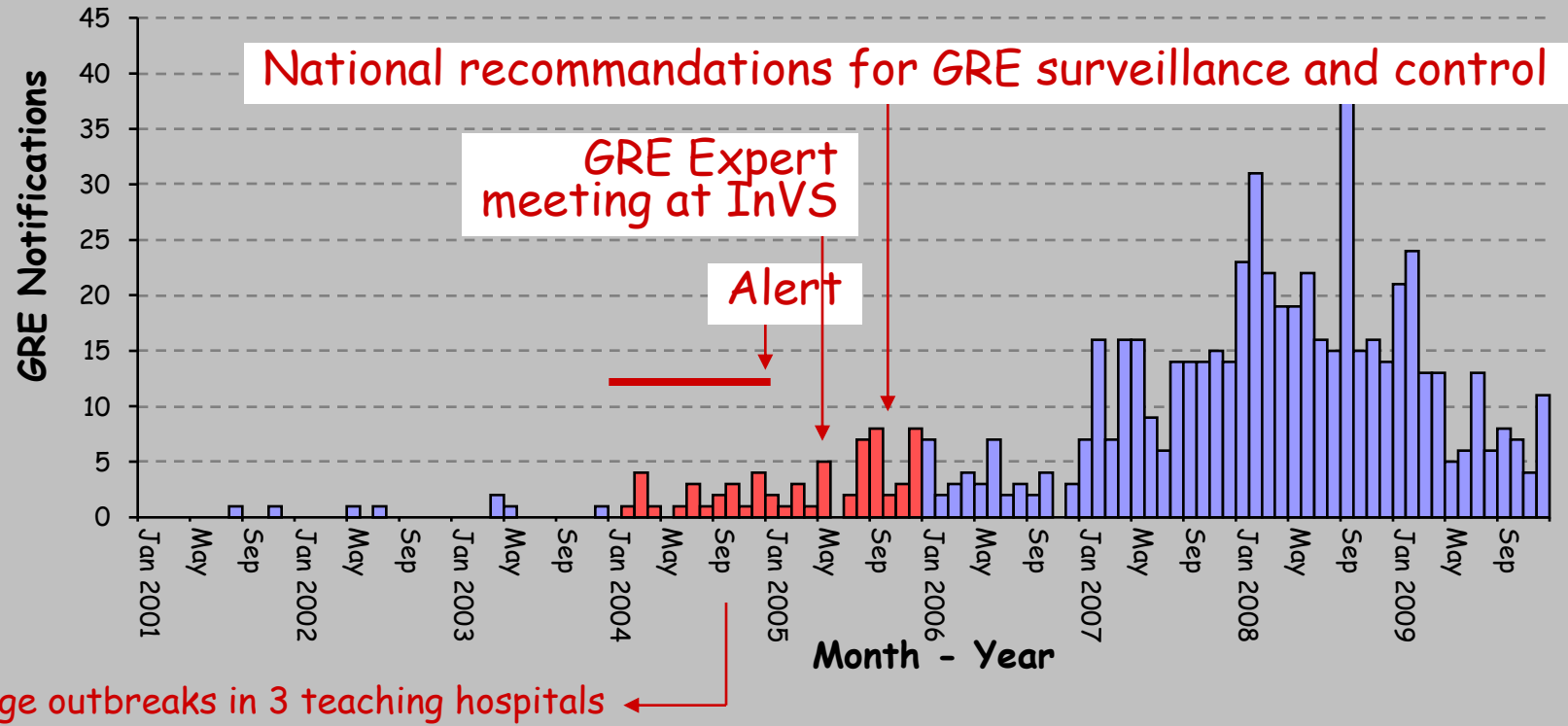
Debate—Guidelines for control of glycopeptide-resistant enterococci (GRE) have not yet worked

J.E. McGowan*

Journal of Hospital Infection (2004) **57**, 281-284

- Failure of control in several countries, e.g., in the US
- However, such guidelines were disseminated only in 1995: more than 5 years after VRE emergence: too late?
- Not systematically applied in all healthcare facilities
- Other countries should benefit from the experience of countries which faced outbreaks earlier

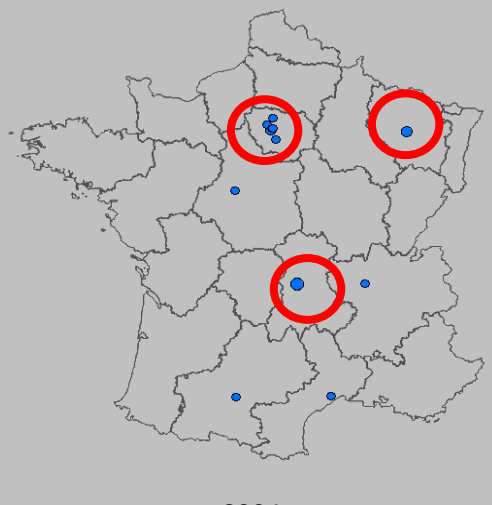
Early Warning: The Detection of VRE Emergence in France, 2004 - 2005



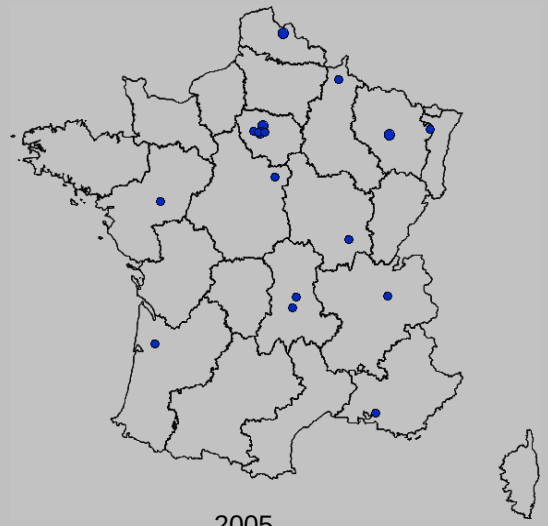
GRE notifications = GRE events
- a single case of colonisation or infection
- an outbreak

Source: InVS/Raisin (http://www.invs.sante.fr/raisin/)

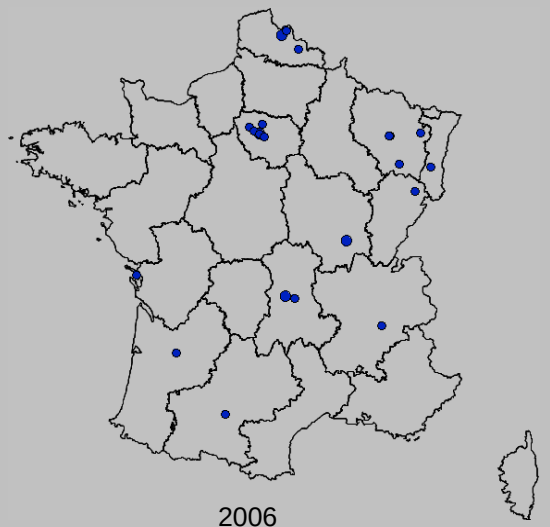
VRE outbreaks



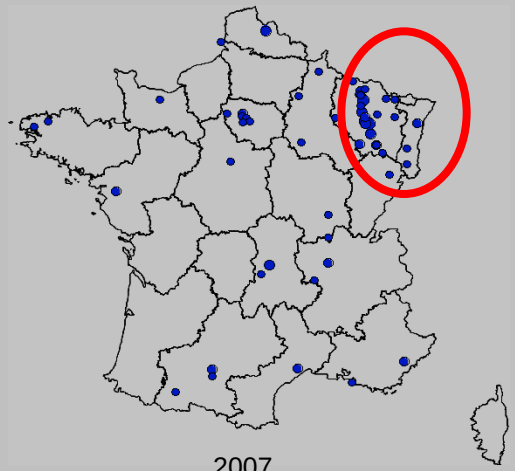
2004



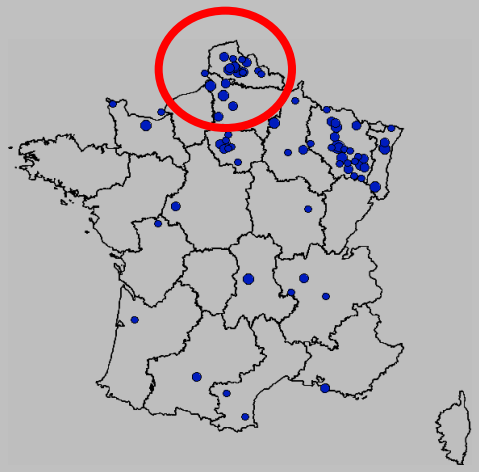
2005



2006



2007

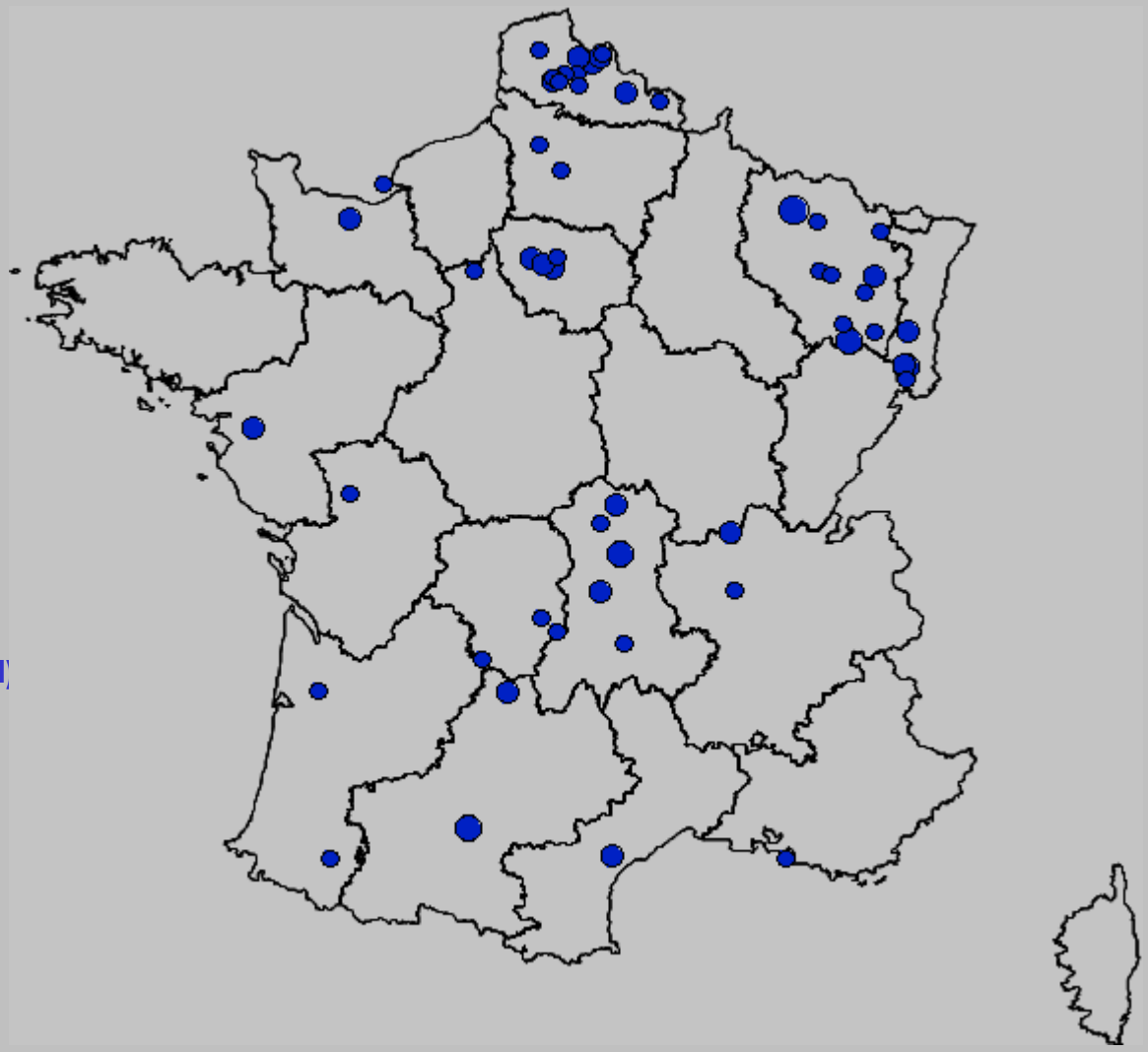


2008

GRE Notifications (N)

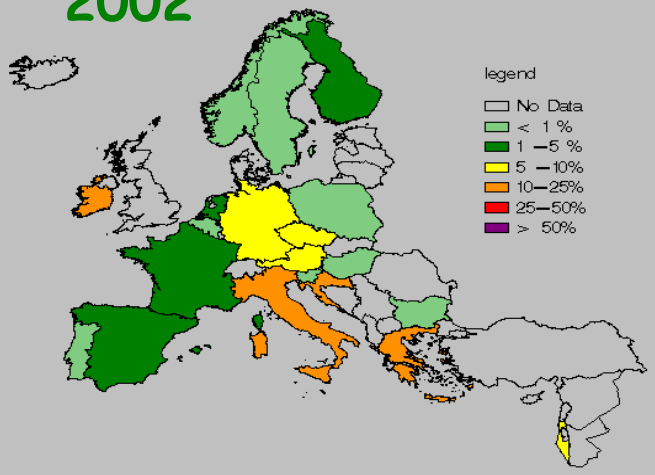
- 1
- 2 - 4
- 5 - 9
- 10 - 15
- 16 - 49

GRE Notifications, France, 2009

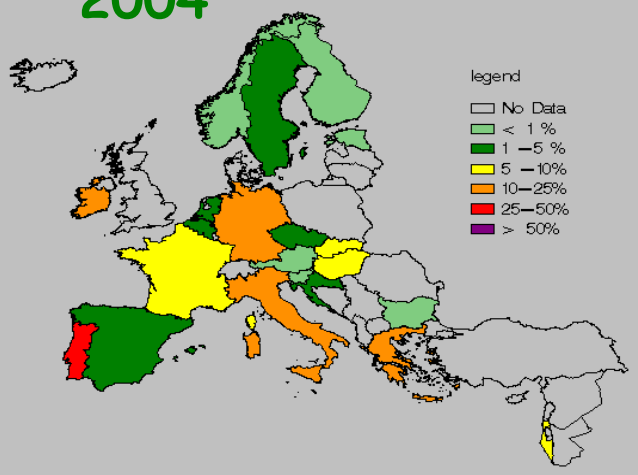


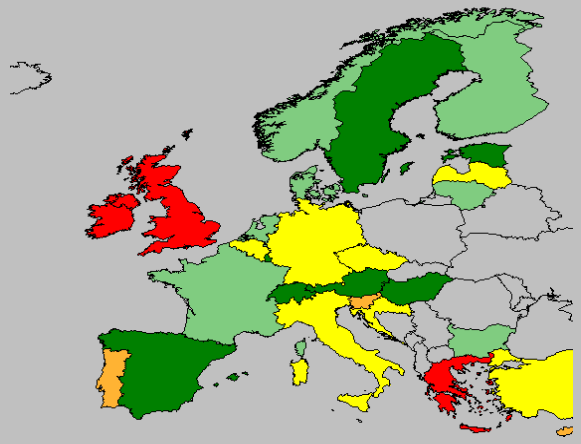
E. faecium in Europe: % VR *E. faecium* in blood cultures (EARSS http://www.earss.rivm.nl/)

2002



2004





2008

Strategy for controlling VRE

- After identification of the first clinical case, screening in stools (rectal swabbing) of contact patients

- Contact precaution for all patients in the unit and if more than one case, cohorting.

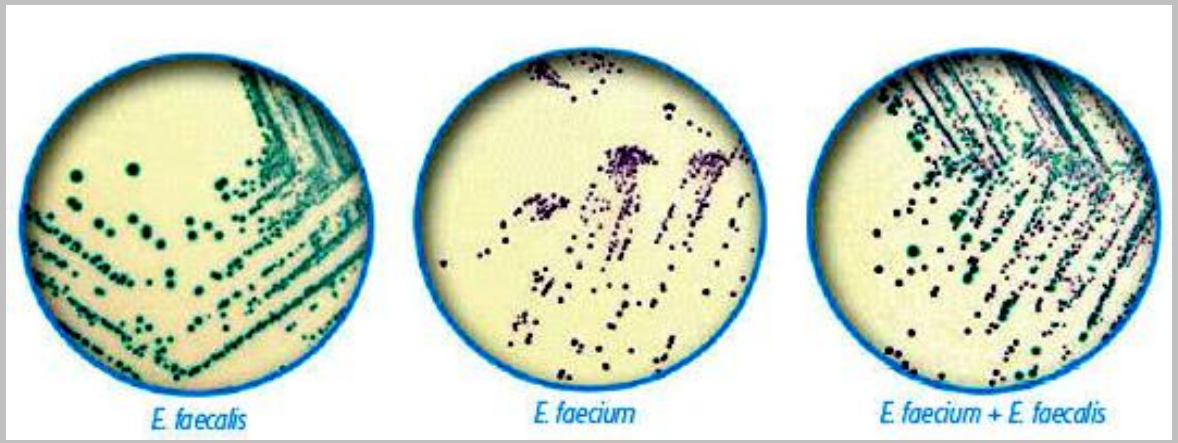
Detection of VRE carriers

- Requirements
  - Rapidity (detection of carriers as soon as possible)
  - Specificity, sensitivity of techniques
  - Cost (cost vs benefits)

Chromogenic media

Without enrichment: detection in 24h-48h

With enrichment: more sensitive (20-30% additional positives detected), but needs 48h-72h.

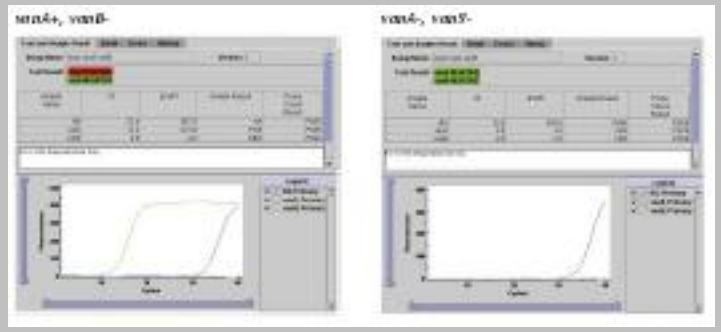


bioMérieux

Real-time detection by real-time PCR: Cepheid Xpert™ VanA/VanB assay

- Fully automated system
- Cartridges Xpert ready-to-go
  - Technique does not require specialized technicians
- Detection of *vanA*, *vanB* genes
  - In 47 minutes :
    - Extraction
    - Purification
    - Amplification
    - Detection

Automated



Cartridges Xpert

Hospital outbreak

- January 2009: 40 cases of VRE in 3 medicine departments (4 infections)
- End of the outbreak : end of February
- Second outbreak episode in March (8 patients) related to readmission of a VRE carrier
- End of the second outbreak April 2009
- Screening campaign: all hospitalized patients in departments at risk for VRE

Screening campaign

- Chromogenic media (chromID™VRE, bioMérieux) after enrichment in broth + vancomycin
- Xpert™ VanA/VanB assay
- 804 samples (prevalence study) (2-3 weeks)

Detection of VRE by Xpert™ VanA/VanB assay and from 804 rectal swabs

Cepheid Xpert™ vanA/vanB assay	Culture pos	Culture neg
vanA or *vanB* (+)	11	116
vanA (+)	8	4
vanB (+)	3	112
vanA/vanB (-)	0	677

Bourdon et al., Diagnostic Microbiology and Infectious Diseases, 2010

Sensitivity and specificity

Result	Value (%) of Cepheid Xpert™ *vanA*/*vanB* assay (95% CI)			
	Sensitivity	Specificity	PPV	NPV
vanA or *vanB* (+)	100 (70–100)	85.4 (82.7–87.7)	8.7 (4.8–15.0)	100 (99.3–100)
vanA (+)	100 (62.8–100)	99.5 (98.7–99.9)	66.7 (38.8–86.5)	100 (99.4–100)
vanB (+)	100 (38.2–100)	85.6 (82.9–87.8)	2.6 (0.6–7.7)	100 (99.3–100)

95% CI = 95% confidence interval calculated by the modified Wald method.

Bourdon et al., Diagnostic Microbiology and Infectious Diseases, 2010

Management of VRE outbreak using Xpert™ VanA/VanB assay

- Outbreak of *vanB E. faecium:* October 2008-April 2009
- Cohorting of patients into 3 zones: zone 1- carriers, 2- contacts, 3- admitted.
- Detection of carriers using chromogenic media
- 1,000 patients screened, there were 182 double screenings (PCR and culture)

Cepheid GeneXpert vs culture

	Culture pos	Culture neg
Xpert vanA/vanB +	19	38
Xpert vanA/vanB -	1	121

	Sensitivity %	Specificity %	PPV %	NPV %
Cepheid GeneXpert	95	76.1	33.3	99.1

Cavalié et al., ECCMID, 2010

Poor specificity of *vanA/B* PCR (rectal swabs)

PCR technique	Sensitivity	Specificity
BD GeneOhm VanR	96.6	87
PCR1	92	49
PCR2	92	60
Cepheid GeneXpert	100	85.4

Ballard et al. Antimicrob Agents Chemother. 2005, 49: 77-81
Sloane et al. J Clin Microbiol. 2004, 42:2636-43
Stamper et al. J Clin Microbiol. 2007, 45:3360-5
Bourdon N et al. Diagn Microbiol Infect Dis. 2010;67:291-3.

Role of PCR in VRE control

- High negative predictive value of Cepheid PCR for VRE screening
- In an outbreak situation: a negative result may be obtained in less than one hour, 24h/24h, and thereby limits the isolation of new admitted patients
- The higher diagnostic cost is balanced by the cost of isolation (rooms, material, staff)
- A positive PCR requires a culture to be carried out and keeping the patient in isolation until the result has been obtained.

Conclusion

- **Bad news**
  - Unlimited reservoir of VRE (strains and mobile genes)
  - CC17 is happy with our hospitals.
  - Eradication does not seem possible, so far.
  - Capacity of *van* genes to disseminate in a variety of hosts (staph?)
- **Good news**
  - VRE can be controlled
    - Early detection and control is a key issue: the sooner, the better!
    - Easier in countries with low VRE prevalence
    - Fully automated PCR is an inovative and effective tool part of the global infection control strategy for VRE

Thank you

- French Institute for Public Health Surveillance (InVS) and Healthcare-Associated Infection Alert, Investigation and Surveillance Network (Raisin)
  - InVS : B. Coignard, JM. Thiolet, I. Poujol, D. Rahib, S. Maugat
- National Reference Centre for Antimicrobial Resistance, Enterococci Associate Laboratory
  - M. Fines-Guyon, M. Fines-Guyon
- And above all, Healthcare Professionals from Infection Control Units, Laboratories, Clinical Wards

trocar TRANSLATIONAL RESEARCH ON COMBATING ANTIMICROBIAL RESISTANCE

SEVENTH FRAMEWORK PROGRAMME