



ESGARS

ESCMID STUDY GROUP
FOR ANTIMICROBIAL
RESISTANCE SURVEILLANCE

European Society of Clinical Microbiology and Infectious Diseases

Surveillance of Gram-positives with low level resistance mechanisms

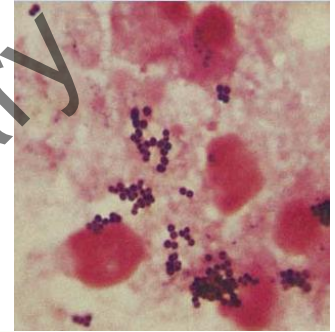
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Which resistance in which Gram-positives?



- Streptococci
- Staphylococci
- Enterococci
- Listeria
- Clostridium
- Bacillus
- Corynebacterium
- Mycobacterium

- Beta-lactams
- Glycopeptides
- Macrolides
- Specific drugs (metronidazole, isoniazid and rifampicin)
- Others?

Specific MDR bugs to survey?

- MRSA: methicillin-resistant *Staphylococcus aureus*
- MRSE: methicillin-resistant *Staphylococcus epidermidis*
- VRE: vancomycin-resistant enterococci
- GRE: glycopeptide-resistant enterococci
- GISA: glycopeptide-intermediate *S. aureus*
- VISA: vancomycin-intermediate *S. aureus*
- VRSA: vancomycin-resistant *S. aureus*
- PRP: Penicillin-resistant pneumococci
- PIP: Penicillin-intermediate pneumococci
- MDR-TB: multidrug-resistant *M. tuberculosis*

Specific MDR bugs to survey?

- **MRSA:** methicillin-resistant *Staphylococcus aureus*
- **MRSE:** methicillin-resistant *Staphylococcus epidermidis*
- **VRE:** vancomycin-resistant enterococci
- **GRE:** glycopeptide-resistant enterococci
- **GISA:** glycopeptide-intermediate *S. aureus*
- **VISA:** vancomycin-intermediate *S. aureus*
- **VRSA:** vancomycin-resistant *S. aureus*
- **PRP:** Penicillin-resistant pneumococci
- **PIP:** Penicillin-intermediate pneumococci
- **MDR-TB:** multidrug-resistant *M. tuberculosis*

MDR bugs to survey for which antibiotics ?

- MRSA, MRSE => *resistance* to **beta-lactams*****
- VRE, GRE, VISA, GISA, VRSA => *resistance* to **glycopeptides*****
- PRP, PIP => *resistance* to **beta-lactams*****
- MDR-TB => *resistance* to **at least isoniazid and rifampicin***

* low or high level resistance

** various levels of cross resistance among the family

What is low level resistance mechanism?

- Means a resistant pattern
- Natural or acquired mechanism of resistance : resistance gene or mechanism
- **Moderately high** MICs, not far from antibiotic breakpoints or in situ concentrations
 - *S. haemolyticus* and teicoplanine (natural)
 - *S. pneumoniae* and penicillin (acquired)
 - Staphylococci and cephalosporins (acquired in *mecA* strains)

>> Categorization “ I or R “
probability of therapeutical failure ?

Detection of the resistance gene

MDR-Gram +	Resistance gene
MRSA, MRSE	<i>mecA</i> (PBP2a)
VRE, GRE VRSA	<i>vanA</i> , <i>vanB</i> <i>vanA</i>
GISA, VISA	Genes involved in cell wall synthesis
PRP, PDSP	Mosaic <i>pbp</i> genes and mutations (<i>pbp1a</i> , <i>2b</i> , <i>2x</i>)
MTB-DR	Mutations in <i>katG</i> and <i>rpoB</i>

⇒ ***mecA*, *vanA*, (*vanB*) detection + identification**

Focus on

PRP

MRSA

VRE

S. pneumoniae and penicillin

**Screening by oxacillin 1 µg disk
diffusion**

Diameter < 18 mm

**AND Identification of *Streptococcus
pneumoniae***

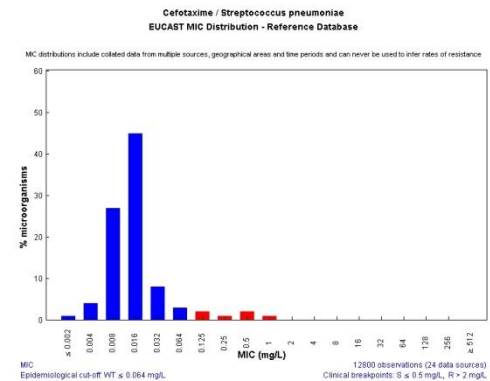
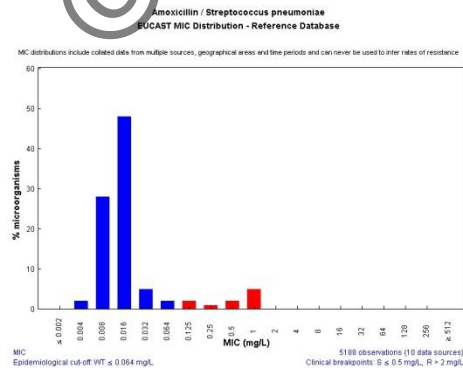
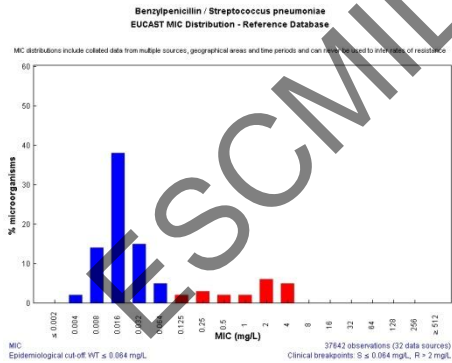
**= suspicion of Penicillin resistant
pneumococci**

=> Determination of MICs

Phenotypic detection of PRP

Identification of *Streptococcus pneumoniae* AND

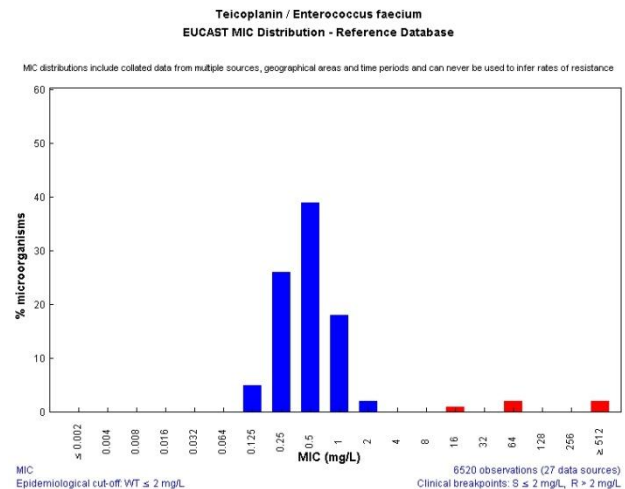
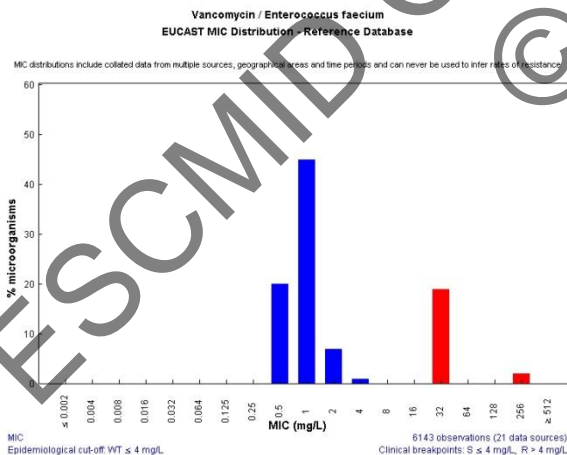
	MIC breakpoints		
	S	I	R
penicillin	≤ 0.06	0.12 - 1	> 1
amoxicillin	≤ 0.5	1 - 2	> 2
Cefotaxime ceftriaxone	≤ 0.5	1 - 2	> 2



Enterococci and glycopeptides

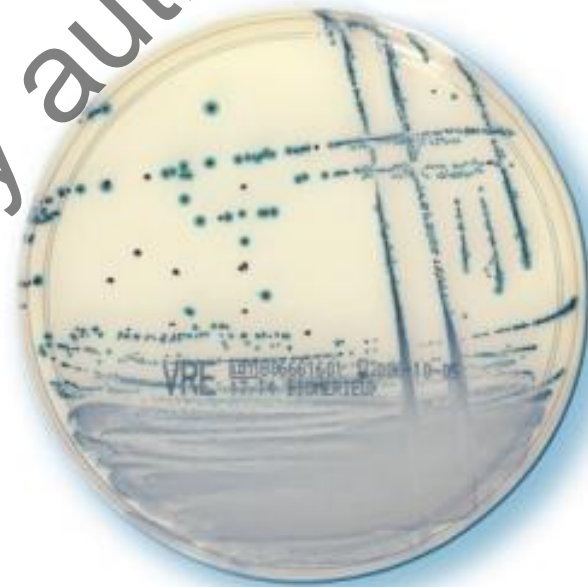
Identification of *Enterococcus faecalis* or *E. faecium* AND vancomycin resistance

	VanA	VanB	VanD	VanE
MIC Vancomycin (mg/l)	>64	4-1000	64	16
MIC Teicoplanin (mg/l)	>32	0,5-2	4	0,5



Screening from rectal swabs or stools

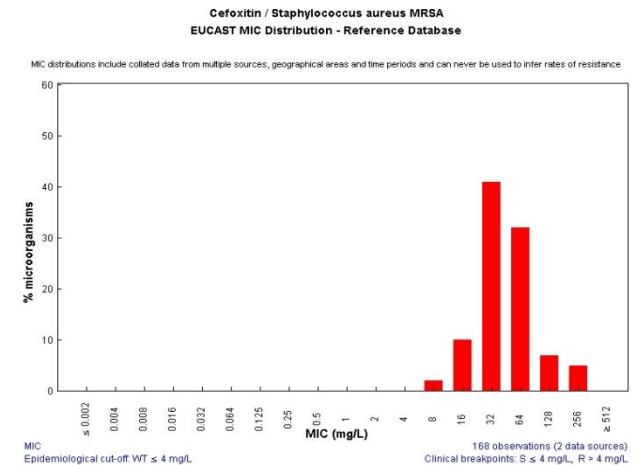
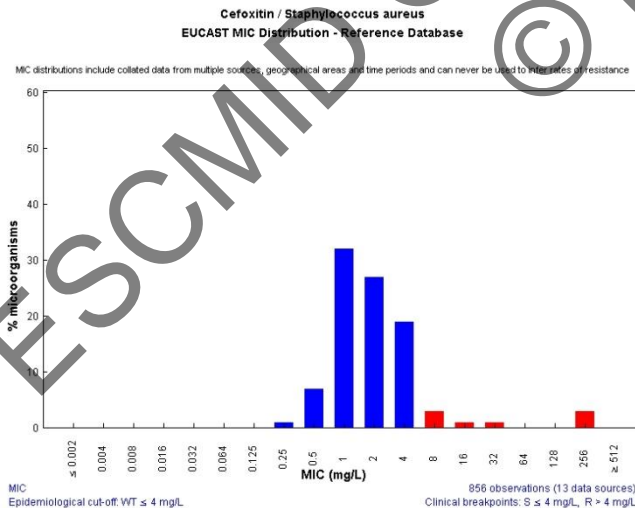
- on vancomycin-containing media
- Chromogenic colonies distinguishing *E. faecalis* and *E. faecium*



Phenotypic detection of MRSA

Antibiotic to be tested	MIC or diameter breakpoints	
Oxacillin	S \leq 2 mg/L	R $>$ 2 mg/L
Cefoxitin 30 μ g disk	S \geq 22* mm MIC \leq 4 mg/L	R $<$ 22* mm MIC $>$ 4 mg/L

* 25 mm for coagulase negative staphylococci or testing moxalactam

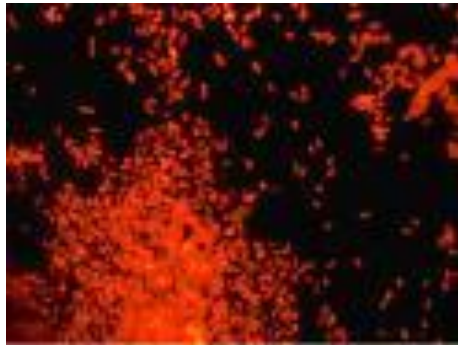


Screening from swabs

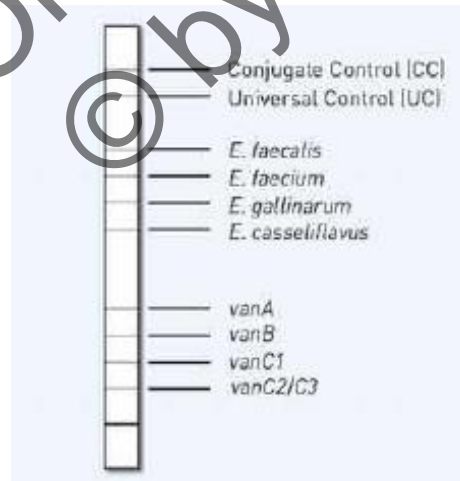
- on cefoxitin-containing media
- Chromogenic colonies of *S. aureus*



How to perform the genotypic detection?



Many commercial kits
With amplification
and/or hybridization



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Example of Tests for detection of MRSA vs MSSA

- StaphLex system => Ssc *mecA*
- GeneOhm => Ssc *mecA-orfX*
- SmartCycler => *mecA-orfX*
- Home PCR => Sa442 and *mecA*
- GenoType => *mecA* and ribosomal RNA genes
- New ones
 - GenXpert MRSA
 - Roche LC
 - Inverness
 - Others coming

80 to 96% SENSITIVITY
90 to 99% SPECIFICITY
1H to 5H
Clinical added value?

**European recommendations for
antimicrobial resistance
surveillance**

CMI 2004, 10:349-383

**G.Cornaglia, W. Hryniewicz, V. Jarlier,
G. Kahlmeter, H. Mittermayer,
L. Strachounski, F. Baquero**

Stratification and epidemiological parameters

- Who is the Patient?
 - Age: child, aging, at work
 - New case or retreated case: antibiotic received if retreated case
 - Country of origin
- Where is he (she)?
 - Home: other patients in the house?
 - Community: school, nursing home...
 - Hospital: length of stay
 - Wards: ICU, surgery, pediatrics....
 - Country of living
- Strain
 - Co-resistance
 - Virulence factors
 - Genotype

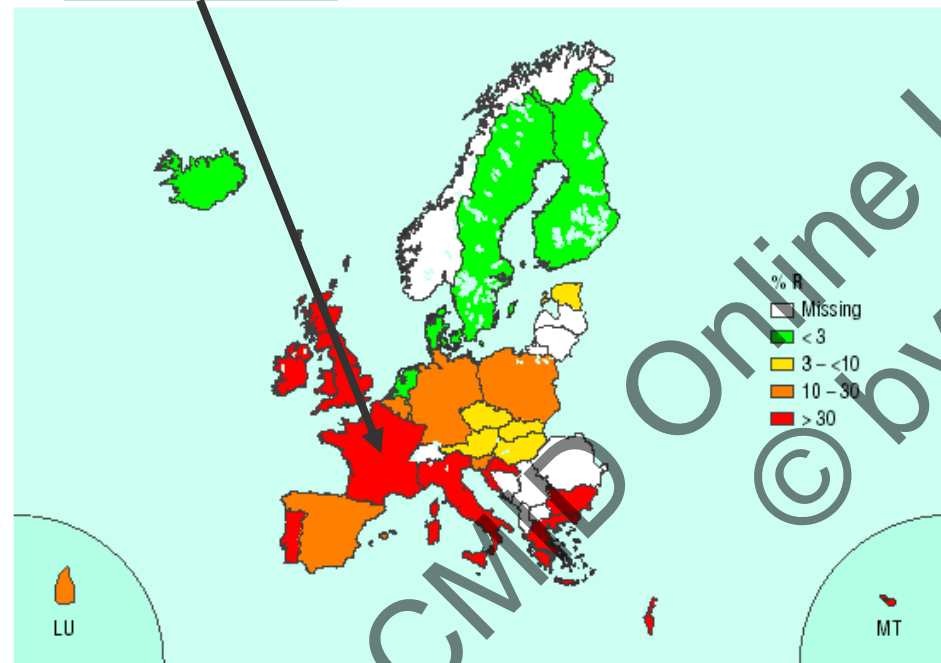
Resistance to penicillin in *S. pneumoniae* according to retreatment and vaccination

		Vaccination	
		Yes	No
Antibiotic receipt in the last 3 months	No	4.2%	10.3%
	Yes	8.6%	16.2%

MRSA in Europe (% in *S.aureus*) according to year (EARSS data)

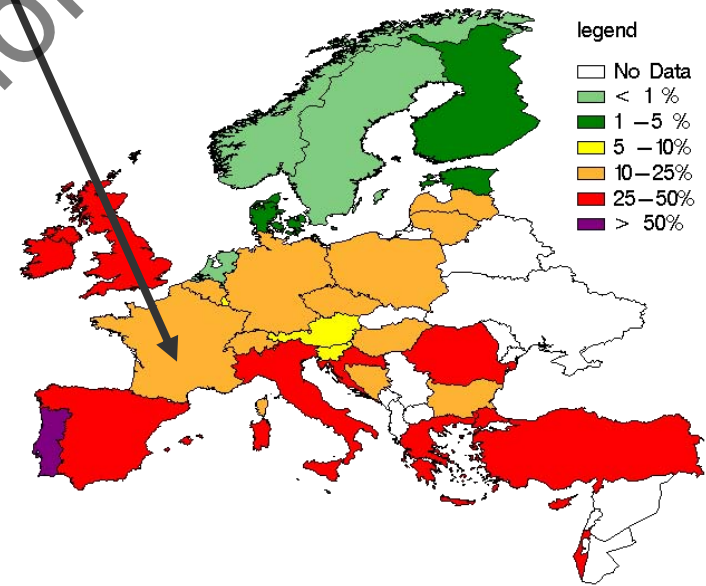
33.2%

24.5%



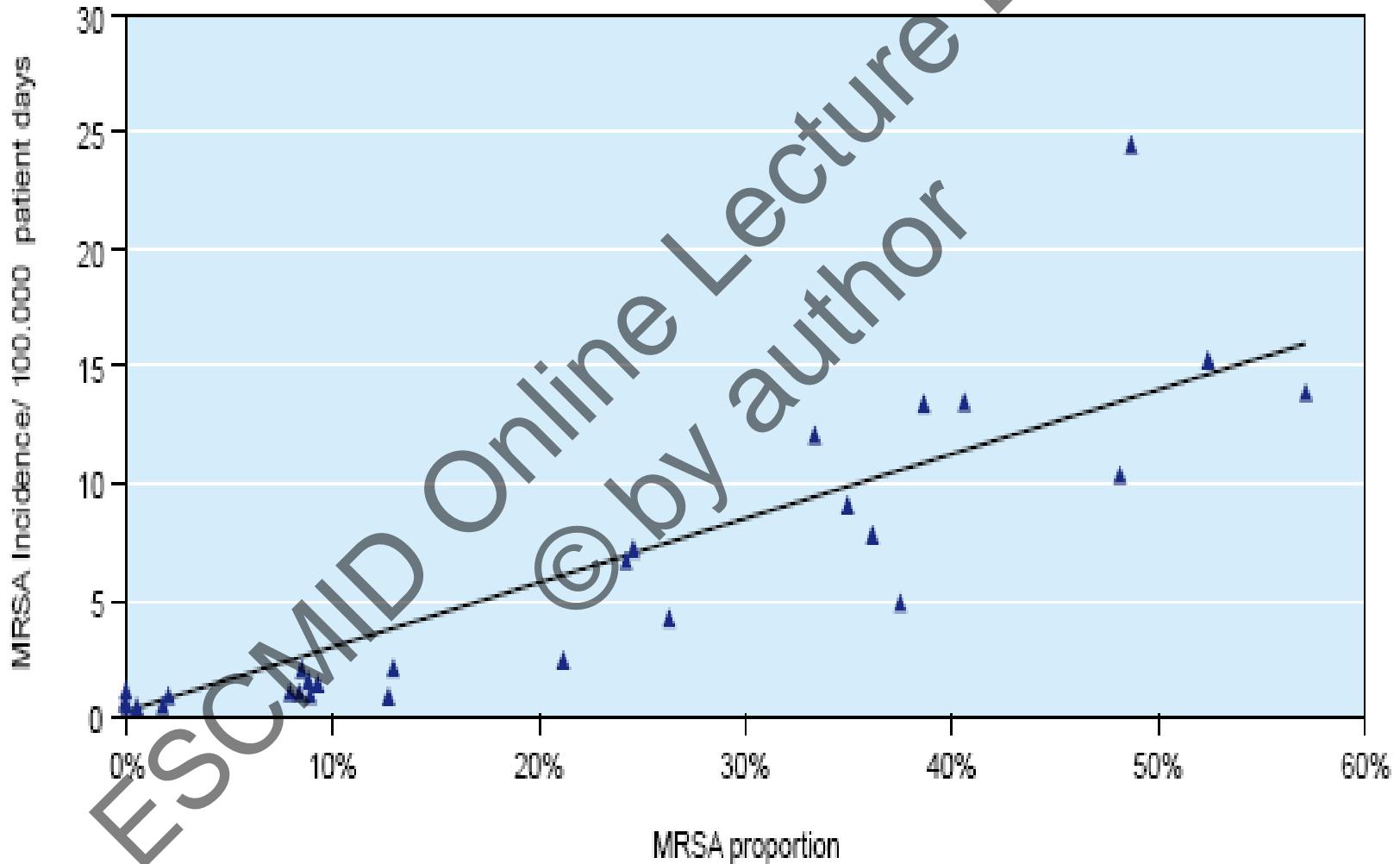
2001

Proportion of MRSA isolates in participating countries in 2008
(c) EARSS



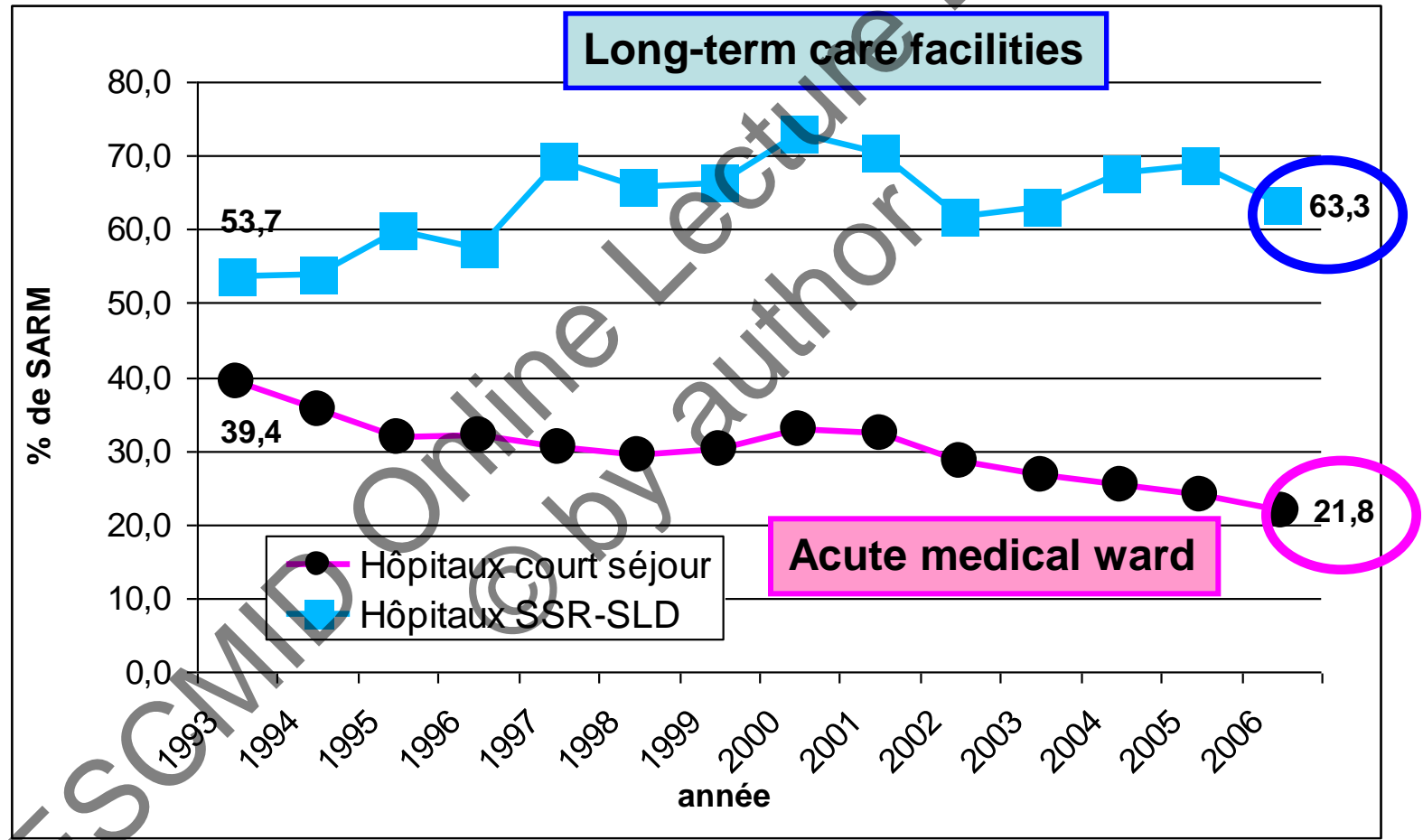
2008

MRSA : proportion among *S.aureus* (%) vs. incidence /100.000 hospital day (EARSS, 2007)



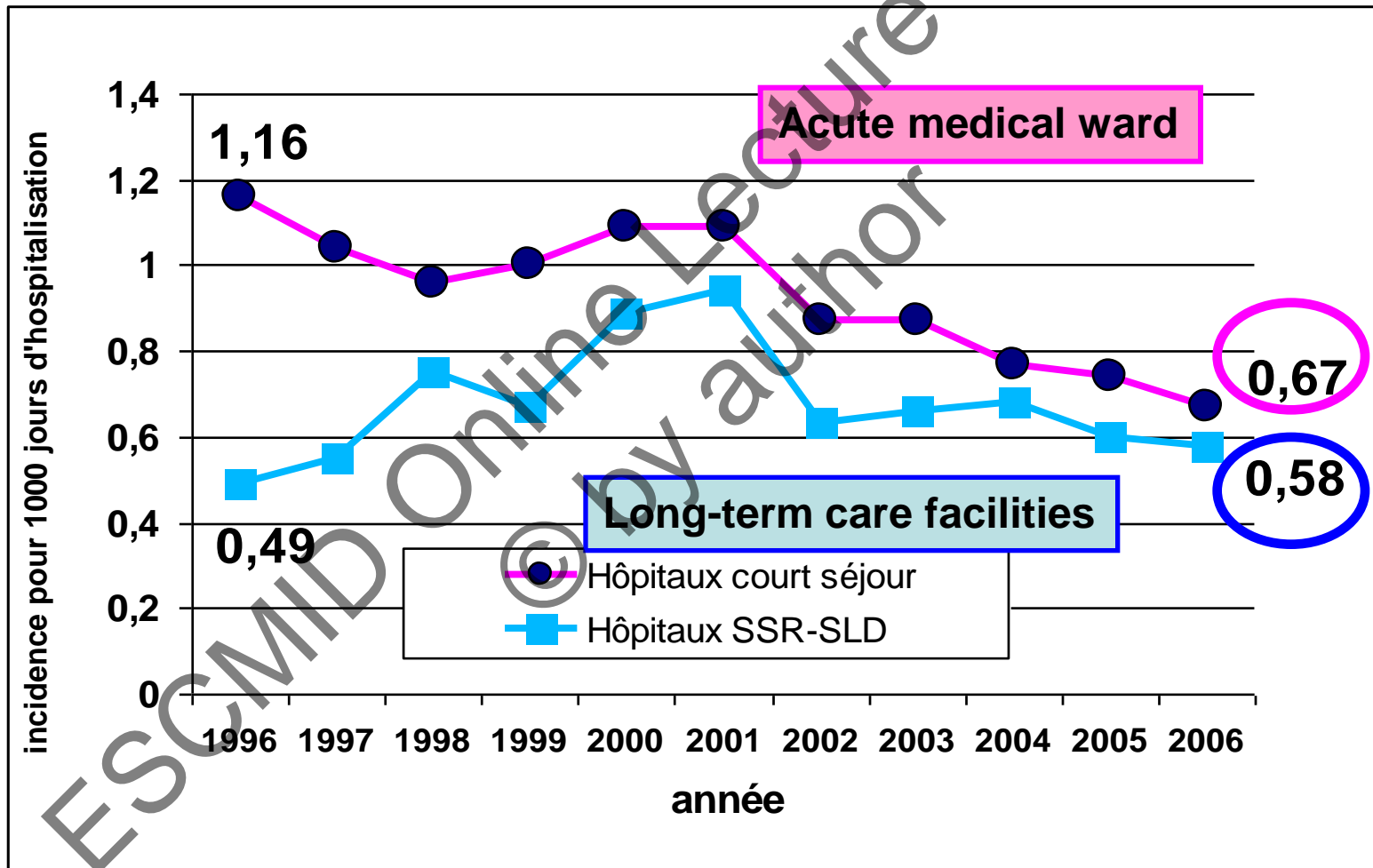
Surveillance data for MRSA in Paris Hospitals

1. Proportion (%) among *S. aureus*



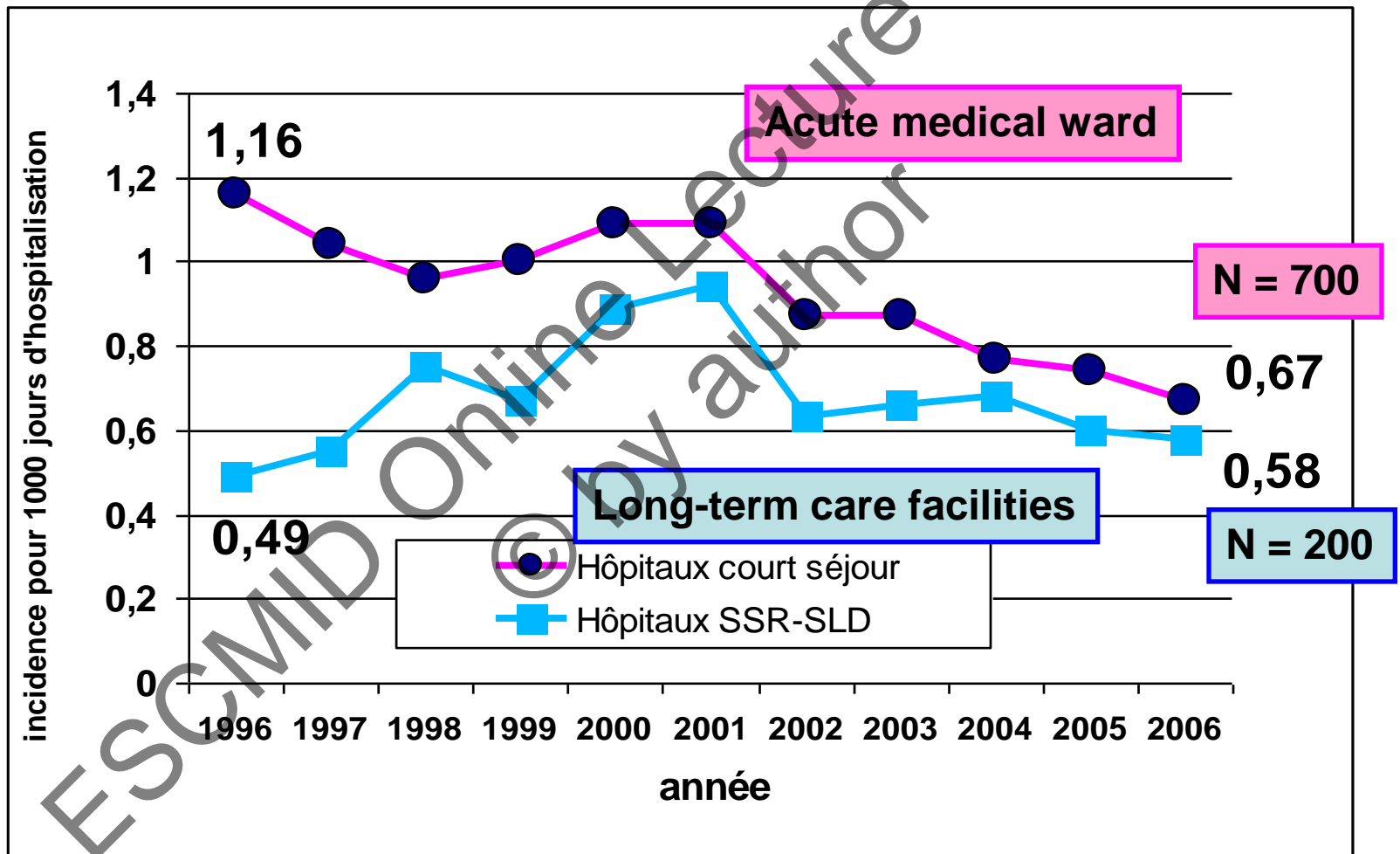
Surveillance data for MRSA in Paris Hospitals

1. Incidence for 1 000 Hospital day



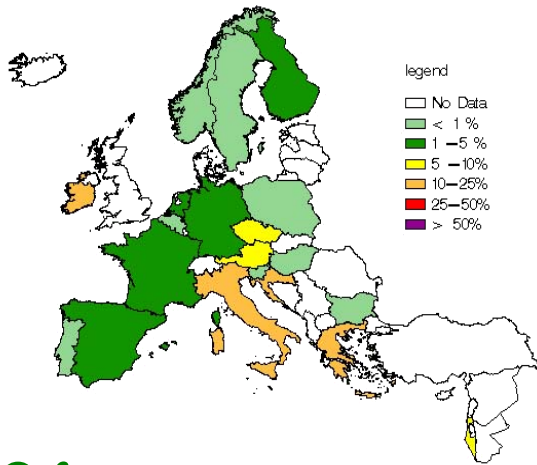
Surveillance data for MRSA in Paris Hospitals

3. Number of cases per year

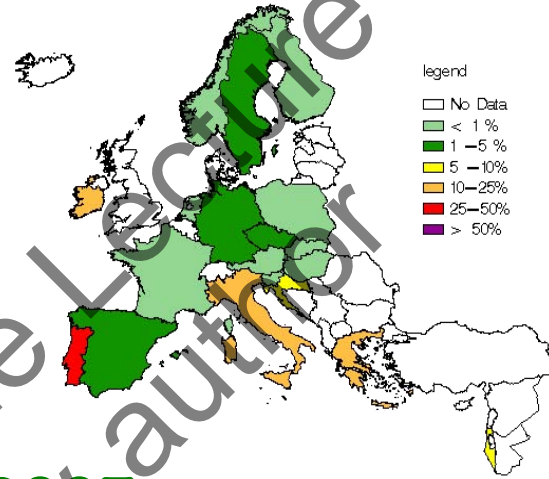


VRE according to year of surveillance

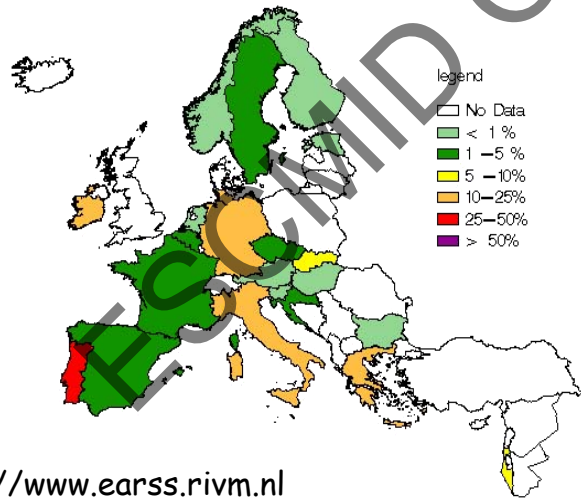
2002



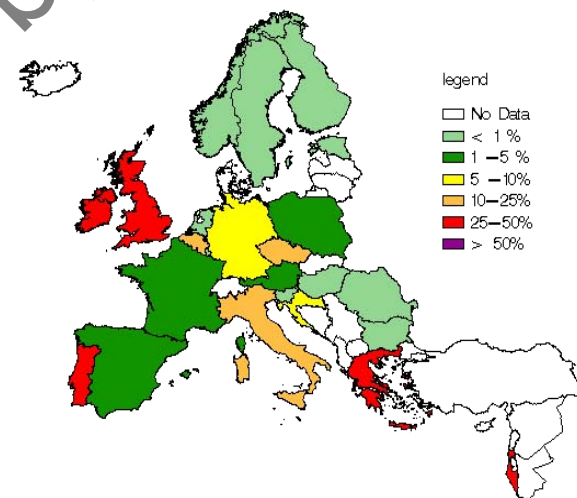
2003



2004



2005



Everyday problems to be solved

(1) MRSA but *mecA* neg

- ⇒ other mechanisms? (BORSA, MODSA, methicillinase)
- ⇒ false negative *mecA* detection?
- ⇒ False resistance with phenotypic method (oxacillin or cefoxitin testing)?
 - Conditions of incubation
 - Purity of the strain
 - Misidentification
 - Antibiotic concentration uncontrolled (old paper disk...)

Everyday problems to be solved

(1bis) *mecA* pos but MSSA

- ⇒ Penicillinase negative strain (*bla*)?
 - ⇒ should retest after induction with beta-lactams
- ⇒ false positive *mecA* detection? How was the negative control of the PCR?
- ⇒ False susceptibility with phenotypic method (oxacillin or ceftoxitin testing)?
 - short incubation, low temperature
 - Should retest in saline medium or at 30°C for 48hours

Everyday problems to be solved

(2) VRE but *vanA* negative

- ⇒ false negative *vanA* detection?
- ⇒ *vanB* detection? Resistance to teicoplanine?
Vancomycin MIC?
- ⇒ False resistance with phenotypic method?
 - Misidentification: *Leuconostoc* (high level resistance),
Enterococcus gallinarum or *E. casseliflavus* (MIC < 32mg/l and
vanC detection)
 - Purity of the strain
 - Antibiotic concentration uncontrolled (old paper disk...)
- ⇒ Other new mechanisms?

Everyday problems to be solved

(3) Pneumococci resistant to cefotaxime but susceptible to penicillin

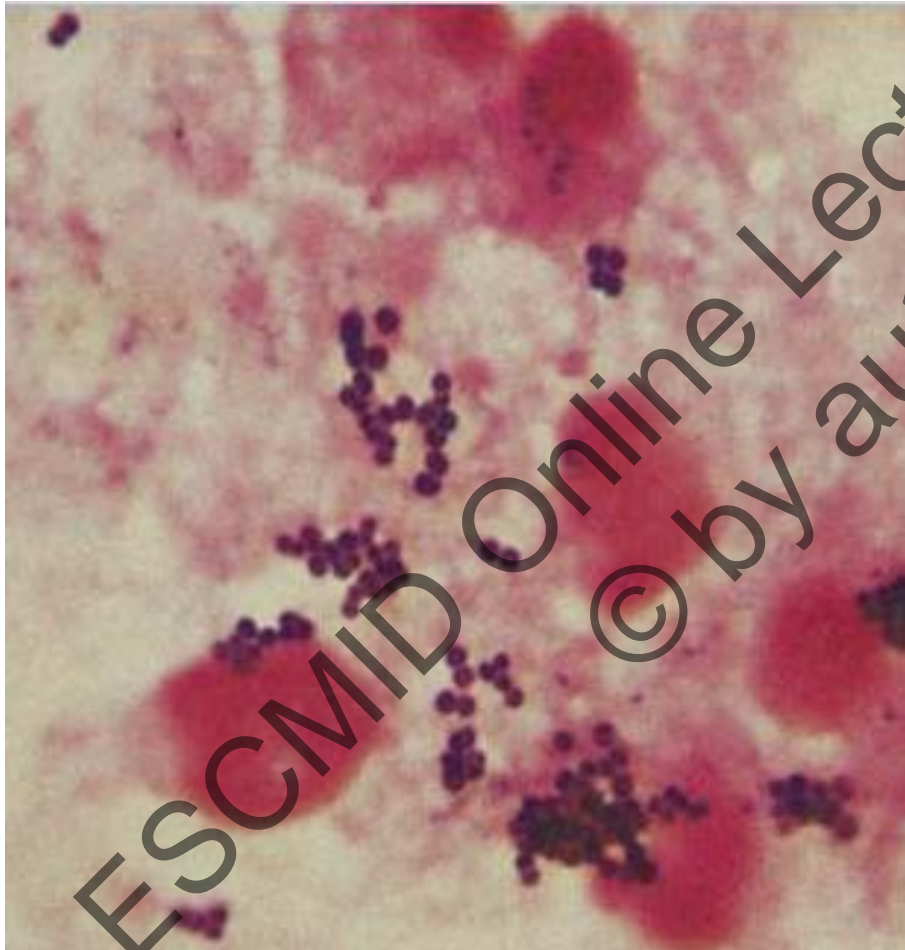
- ⇒ Misidentification: Enterococci, *S. oralis/mitis*
- ⇒ How were the MICs determined :
 - Etest method procedure
 - Purity of the strain
 - Etest strip stayed at room temperature or not in a dry tube
- ⇒ What are the MICs of penicillin, amoxicillin, cefotaxime and ceftriaxone?

Case study

- Two Positive blood cultures at 12.00 h
- With Gram-positive bacteria
- ICU patient, 45 y.o.
- Cardiac surgery 10 days before
- No previous microbiology record



Result of Gram stain of positive blood culture



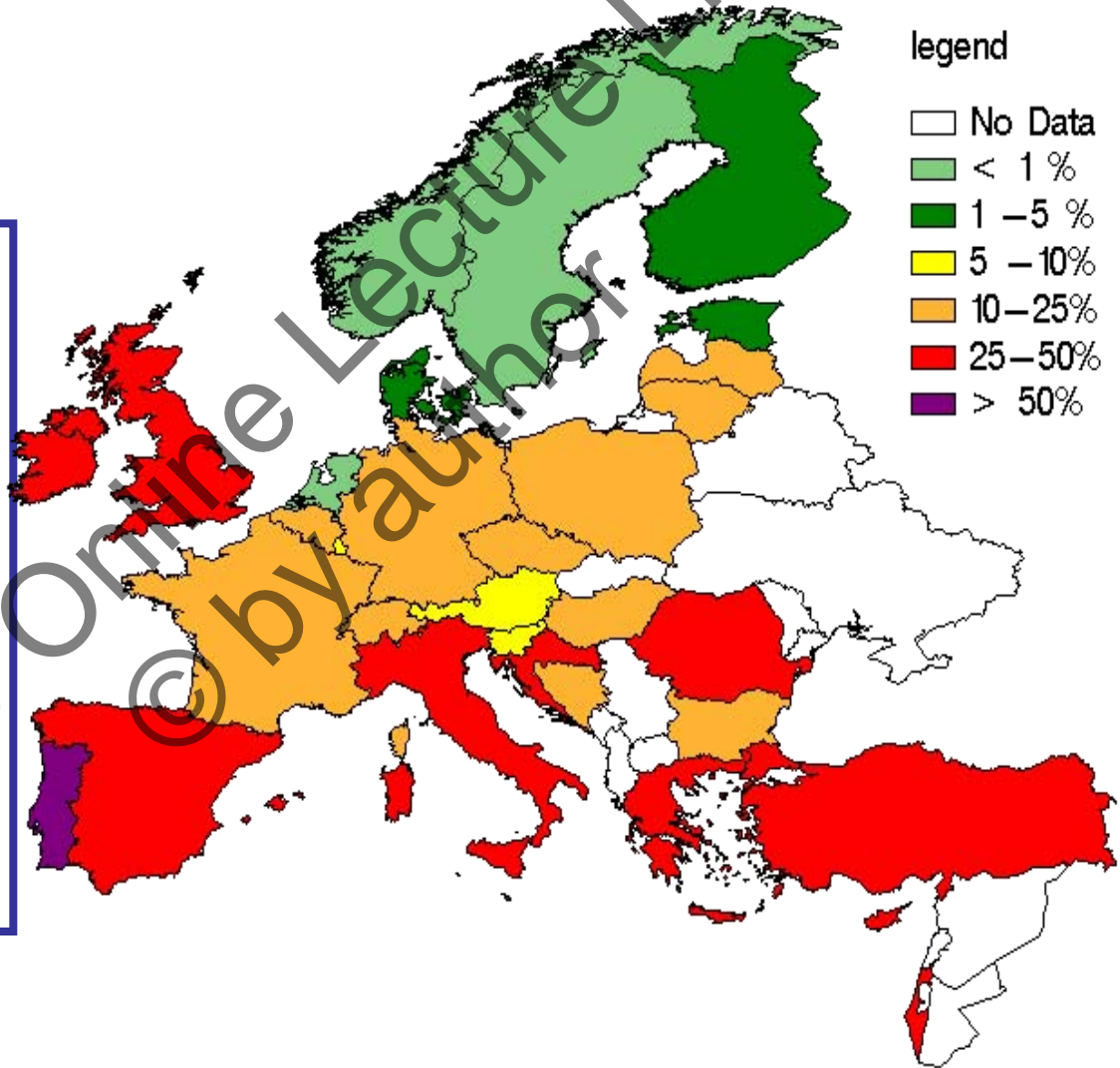
= Gram positive cocci
suggestive of staphylococci

**What will you do
for MRSA
detection ?**

Possible answers to be discussed

1. Rapid detection of *mecA*
2. Rapid detection of *mecA* and a gene specific of *S. aureus*
3. Agar diffusion susceptibility testing with Oxacillin disk 5 µg
4. Culture on blood agar to see hemolysis colonies
5. Culture on agar and secondary disk diffusion or automate liquid susceptibility testing
6. cefoxitin disk diffusion testing

Proportion of MRSA isolates in participating countries in 2008
(c) EARSS



Are you
in Sweden?
in France?
in UK?

My proposals

1. Rapid detection of *mecA*
2. **Rapid detection of *mecA* and a gene specific of *S. aureus***
3. Direct agar diffusion susceptibility testing with Oxacillin disk 5 µg
4. Culture on blood agar to see hemolysis colonies
5. Culture on agar and secondary disk diffusion or automate liquid susceptibility testing
6. **Direct cefoxitin disk diffusion testing**