

New insights from laboratory to classify the affecting and diffusing ability of *Staphylococcus aureus* in rabbit

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So far, the development of rabbit breeding was mostly influenced by:

- rabbit meat supply and demand
- raw material costs
- cost of labor
- energy cost
- growth performance (genetic evolution)
-



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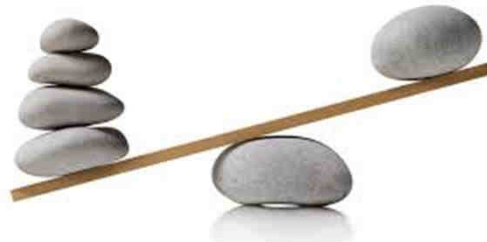


AMR is a worldwide problem and a top priority for Public Health Services



**Public
Health**

Market



The future of rabbit breeding?



Data on antimicrobial consumption in food producing animals are now available to all people

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These amounts of antimicrobial consumption are no more justifiable or acceptable in Europe

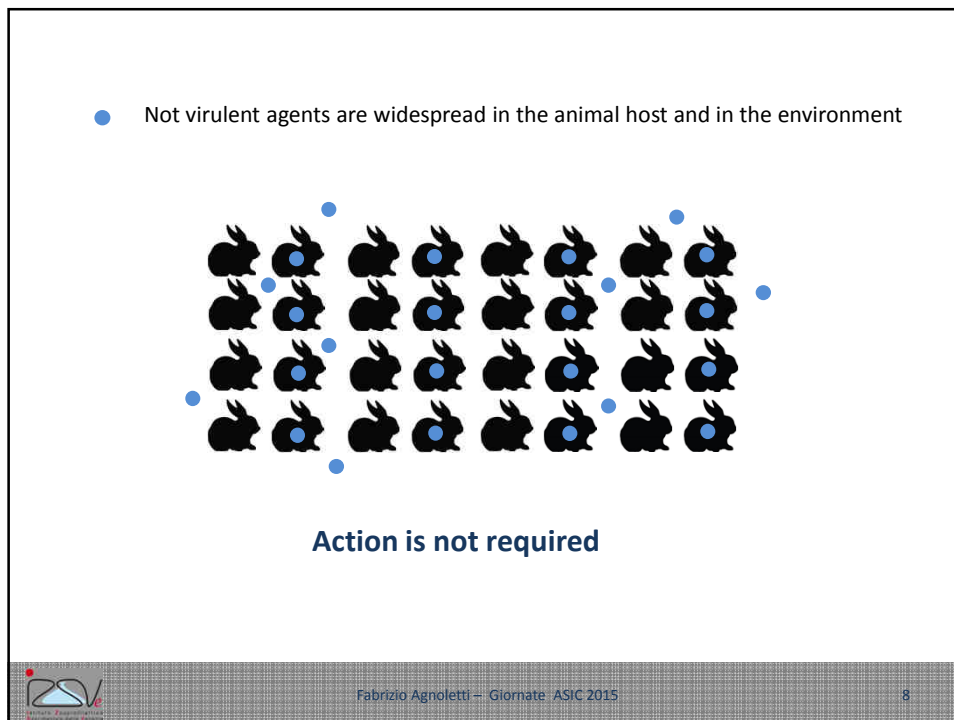
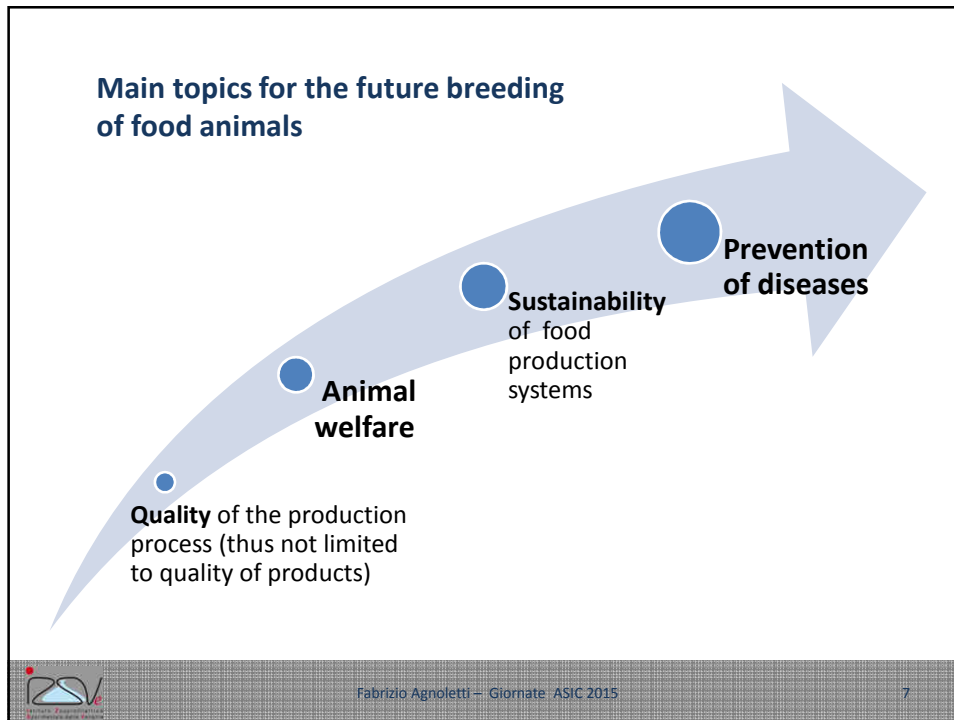
Antimicrobial consumption in 32 rabbit herds in Italy (mg p.a./kg p.v.v. and therapeutic doses)

Year	1	2	1	2	1	2	1	2	1	2
mg p.a./kg p.v.v.	2681,0	2378,9	2468,6	2490,5	2112,1	2048,2	1835,8	1909,6	1814,0	1799,3
dose terapeutica	92,9	87,9	93,3	105,2	78,3	80,8	65,0	62,4		

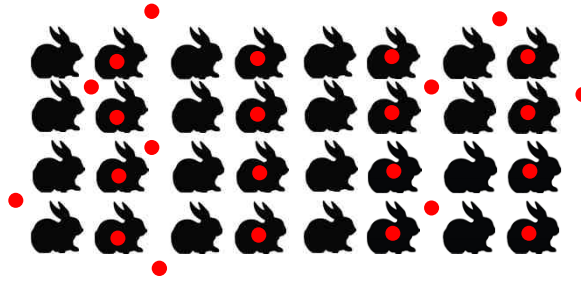
Sales of veterinary antimicrobials in France in 2013

Animal Species	Body weight treated daily / Animal mass (mg p.a./kg p.v.v./dL)
Cattle	~1
Domestic Caprines	~4
Rabbits	~24
Pigs	~5
Poultry	~5

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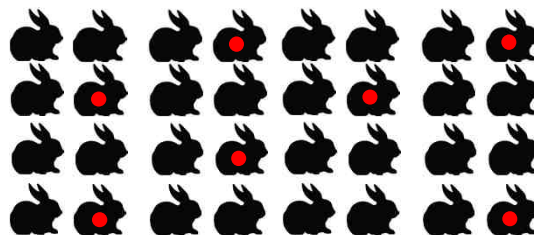
- Virulent agents are widespread in the animal host and in the environment



The only prevention strategy is the vaccination



- Sometimes the virulent agents are present in the animal host

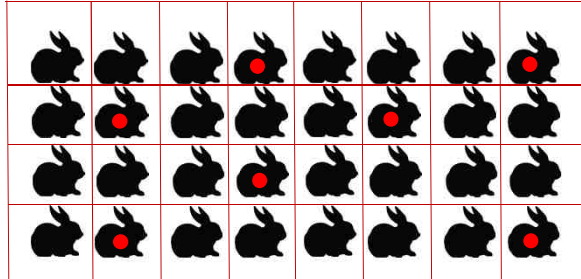


Several prevention and control strategies are available:

- biosecurity and vaccination
- biosecurity and culling



- Sometimes the virulent agents are present in the animal host

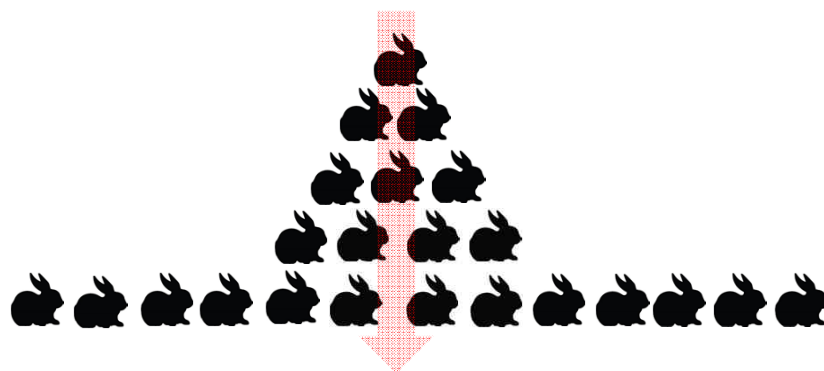


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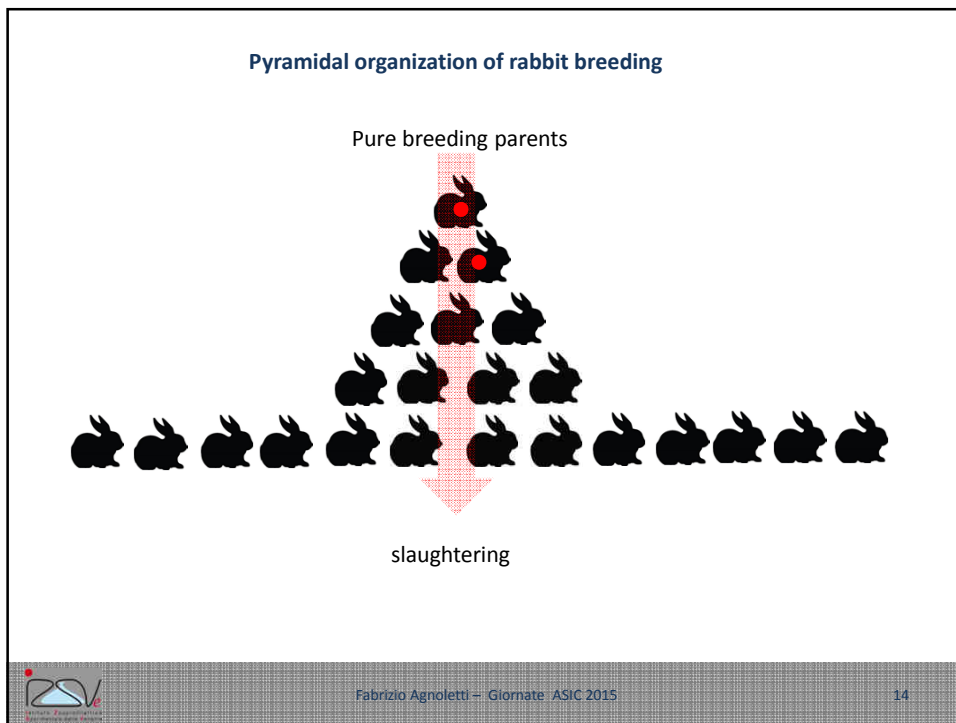
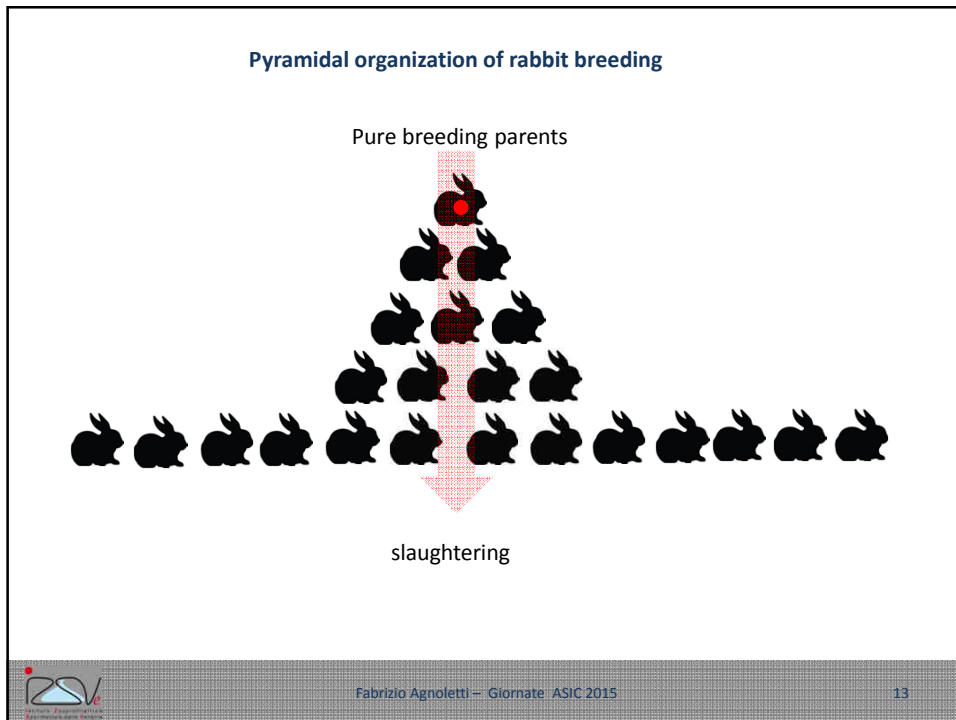


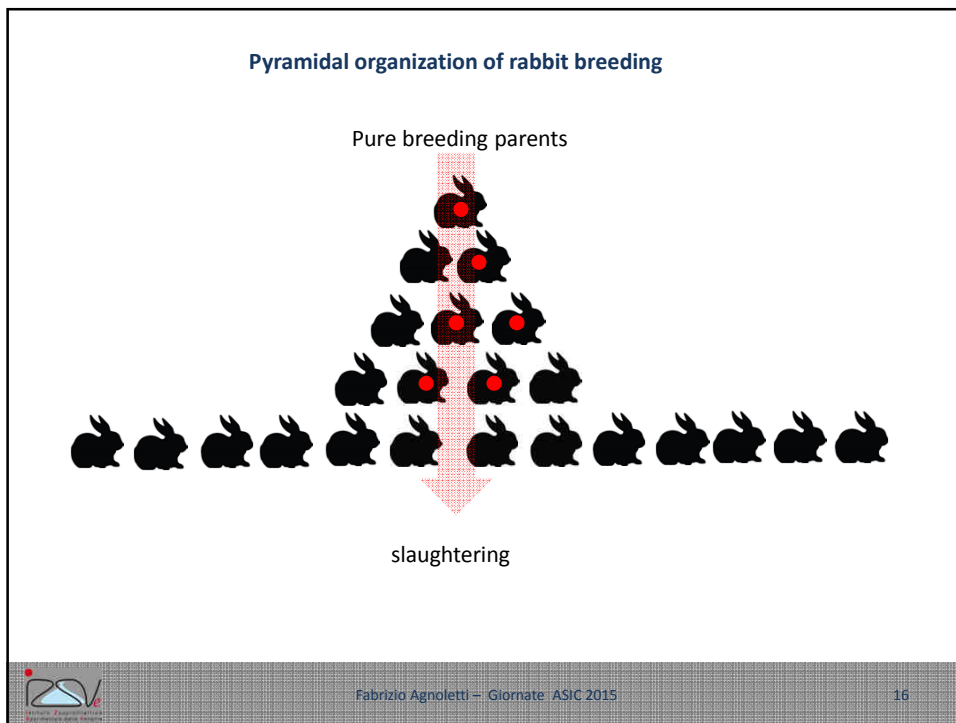
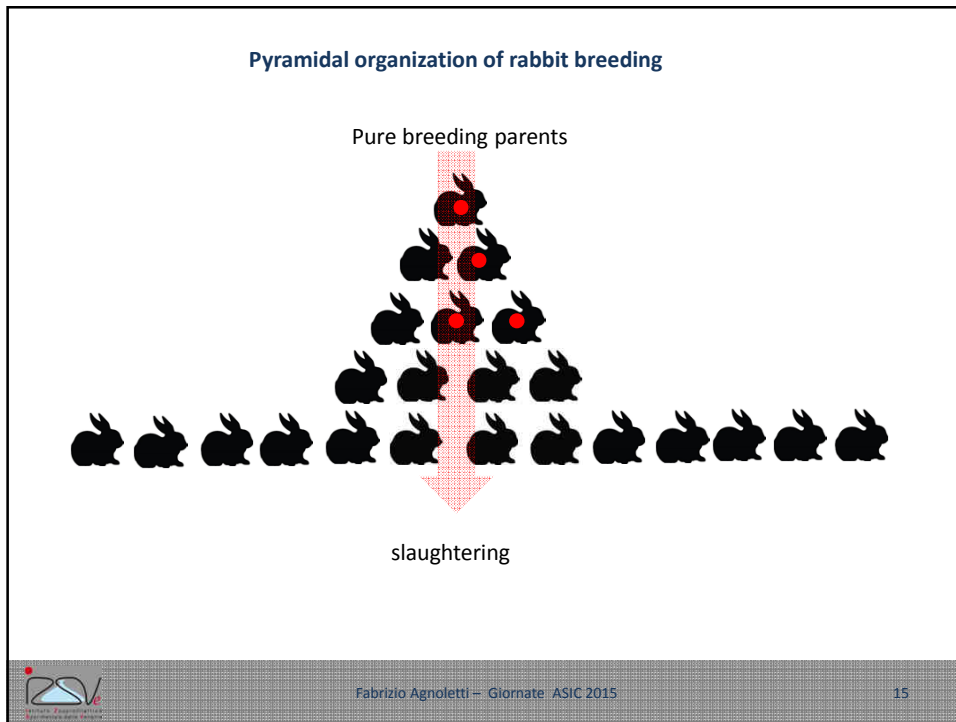
Pyramidal organization of rabbit breeding

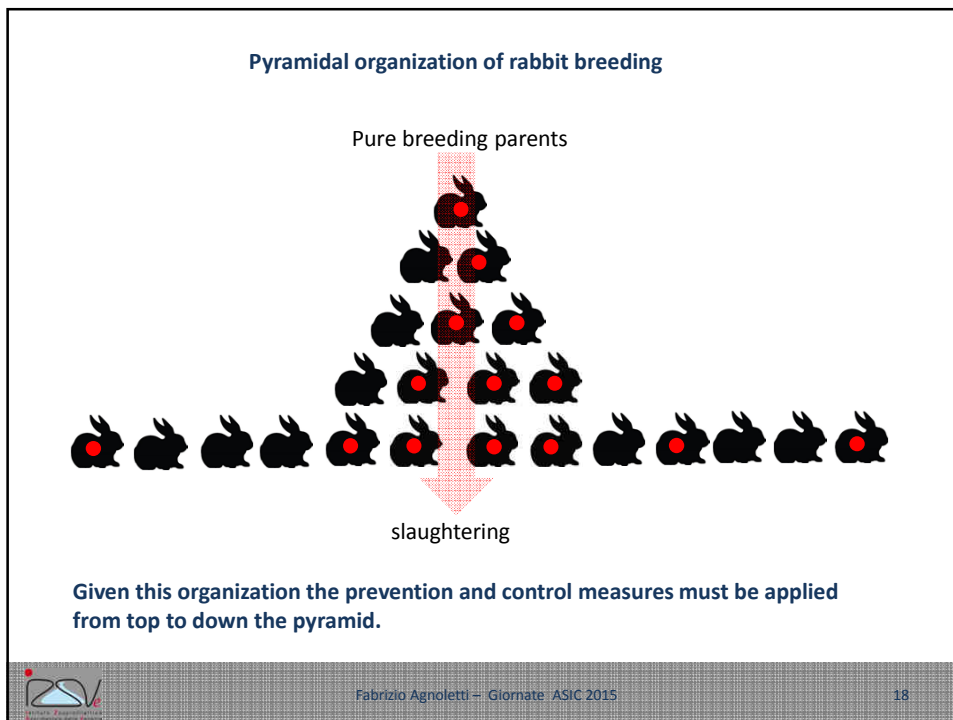
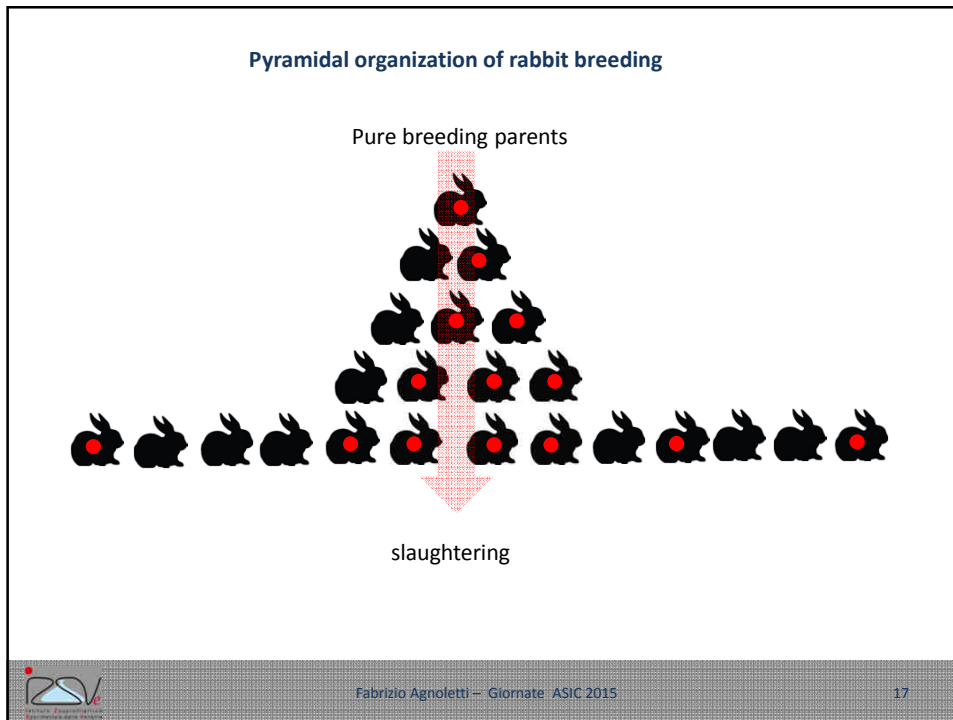


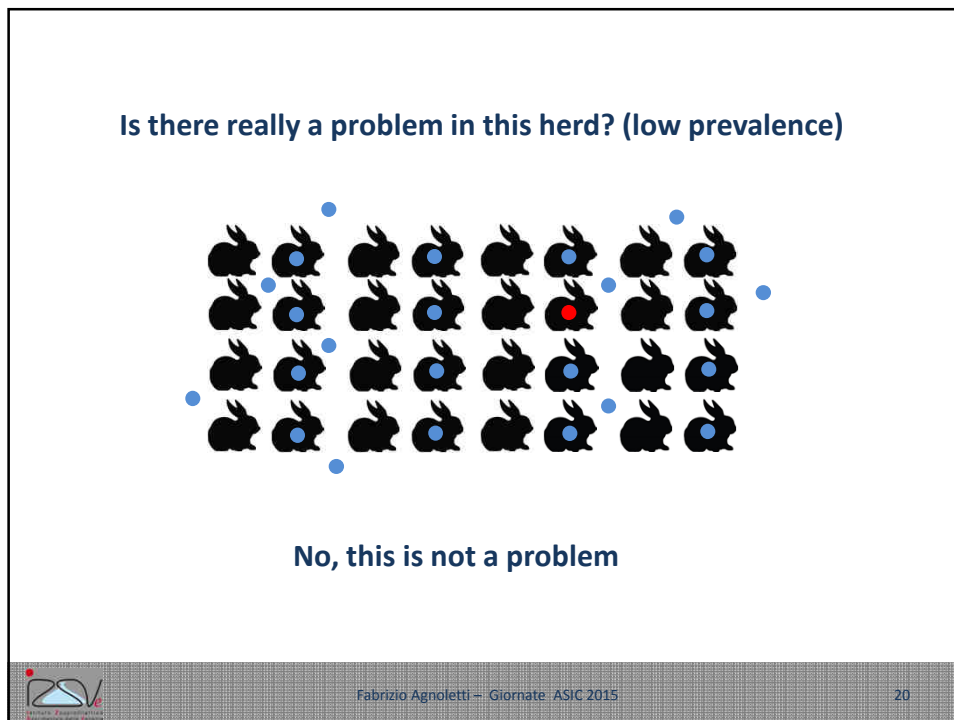
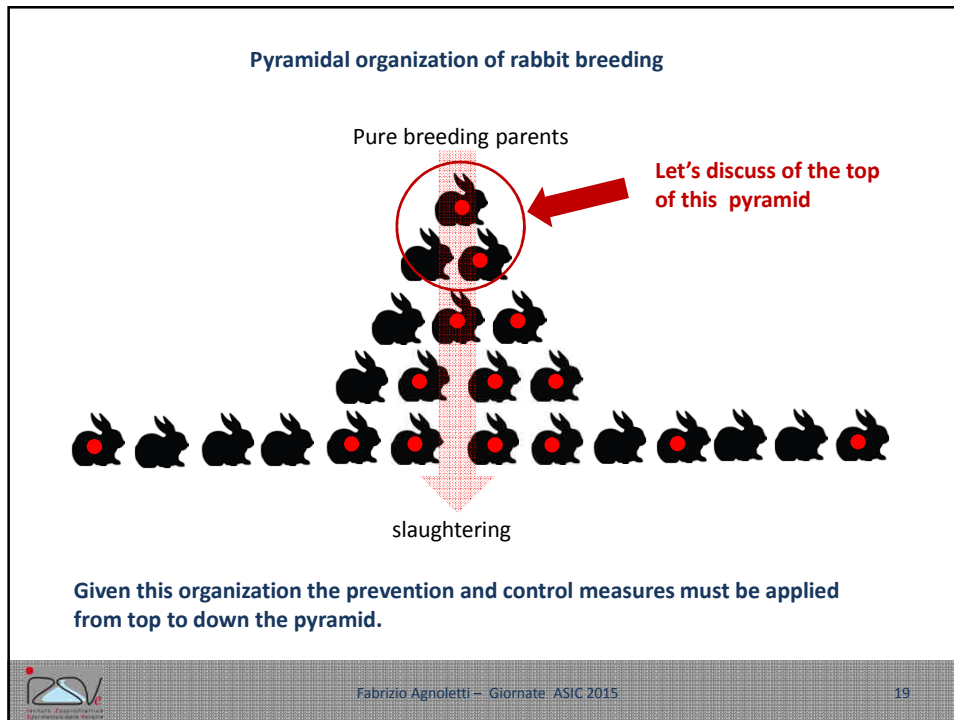
slaughtering



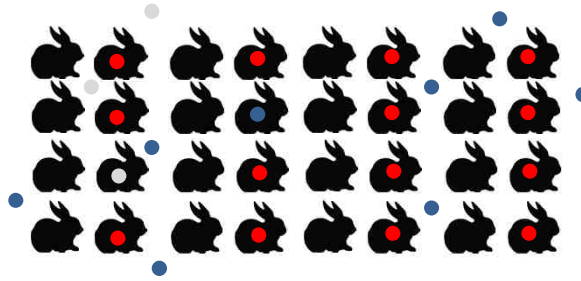








Is there really a problem in this herd? (high prevalence)

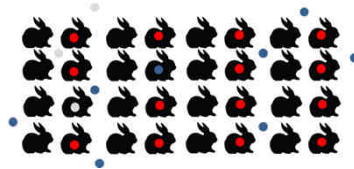


Yes , this is quite a problem!



Virulence in rabbit herds is related to:

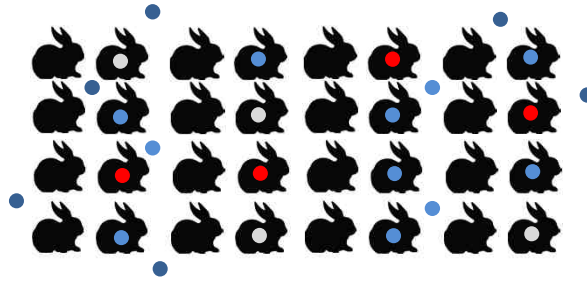
- the ability to affect animals by causing internal or external suppurative lesions
- the ability to diffuse within-flock (thus affecting a high proportion of animals)



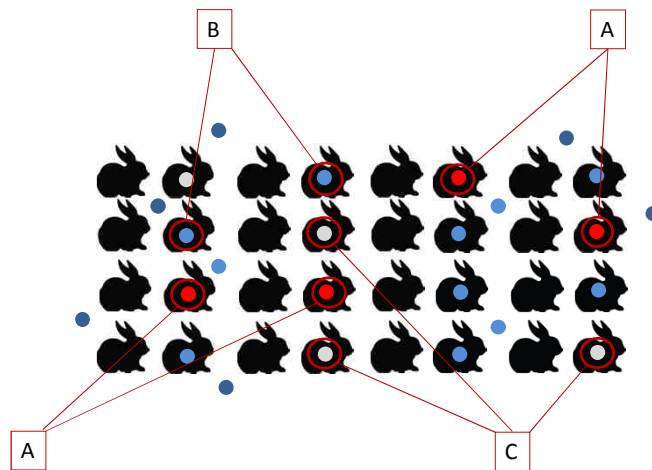
**That's why we propose a new "case definition":
the Affecting and Diffusing *S. aureus***



- Not virulent *S. aureus*
- *S. aureus* with intermediate virulence
- *S. aureus* with high virulence



Mind, there are blue and red bacteria but also gray ones....



Laboratory tests with high discriminatory power are necessary to distinguish *S. aureus* clones with different abilities to affect and diffuse





Before the biomolecular era the phenotypical characterization was based on:

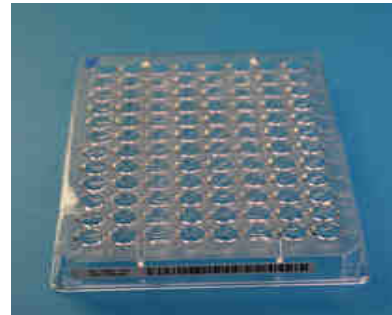
- **serotyping**
- **antibiotyping**
- **biotyping**
- **phagotyping**



The antibiotype

(It's the pattern of antimicrobial susceptibility/resistance mostly tested by MIC)

It varies according to antimicrobial exposure, thus it's not useful for our purposes.



S. aureus biotyping:

- low predictive value
- sometimes misleading (Human= not pathogenic)

BIOTYPE* (ecovar)	β Hemolysis	Growth in cristal violet	Stafilokinasis	Bovine plasma coagulation
HUMAN	-/+	C	+	-
BOVINE	+	A	-	+
OVINE	+	C	-	+
AVIAN	-	A	-	-
(NHS) MIXED CV-C	+	C	-	-
(NHS) MIXED CV-A	+	A	-	-
	+	A	+	-
	-	A	+	-
Not classifiable	-	C	-	-

* Devriese, 1981-1984

C: violet/blue colonies

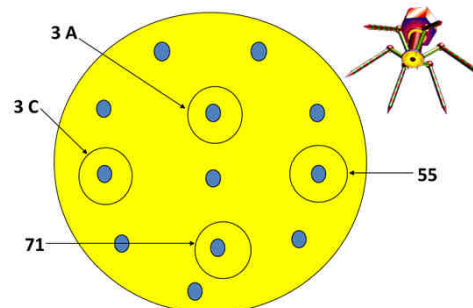
A: yellow colonies

↓
MIXED CV-C



The phage typing

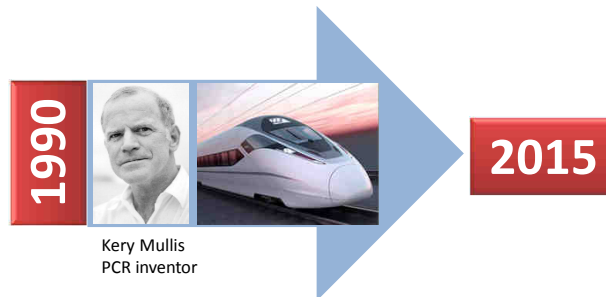
- expensive
- difficult to standardize
- low intralaboratory repeatability



↓
Phage type: 3A/3C/55/71



The evolution of microbiological laboratories: from phenotype to genotype



Molecular methods mostly used for *S. aureus* epityping:

- PCR
- MLST
- PFGE
- *Spa* typing
- MLVA (Multiple-locus variable number of tandem repeat analysis)



S. aureus

- biotype Mixed CV-C
- phage type 3A/3C/55/71

- *bbp+*
- *selm+* (*egc* cluster: *selo*, *selm*, *sei*, *selu*, *seln*, *seg*)
- flank sequence +

Vancaeynest et al. Multiplex PCR assay for the detection of high virulence rabbit *Staphylococcus aureus* strains. *Vet Mic* 121 (2007) 368-372.

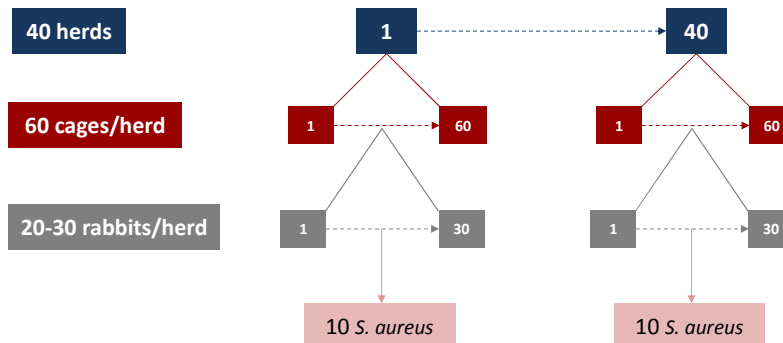


Study aim:

- to verify differences in findings on geographical distribution of *S. aureus* clones of north-european Authors working on a limited dataset
- compared to virulence attribution limited to data from individual rabbits to explore a wider definition of rabbit *S. aureus* virulence linking the rabbit affecting ability to the within-flock diffusing ability, getting inspired from field evidence that a *black and white* classification of staphylococcus, i.e. HV-*S. aureus* vs LV-*S. aureus*, does not represent the entire picture



Our study: the experimental design



Our study: the laboratory analysis

857 *S. aureus*



All strains bio typed



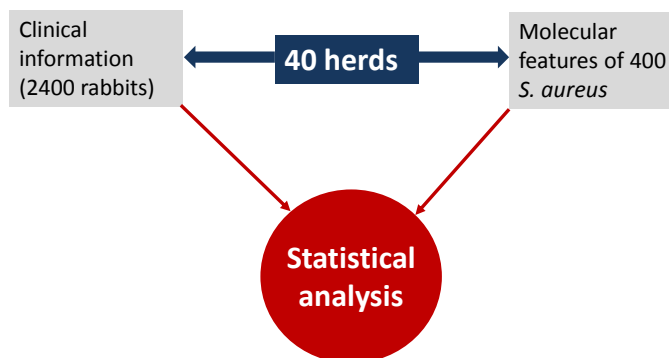
10 strains/herd

- PFGE
- *spa* typing
- MLST

- *fnbB* (fibronectin binding protein B)
- *cna* (collagen binding protein)
- *bbp* (bone sialoprotein binding protein)
- *selm*
- *pvl* (Panton-Valentine leukocidin)
- *flank*
- *mecA*
- *mecC*



Our study: the experimental design



Our study: the results

***S. aureus* is widespread between rabbits (82.4%) and within flocks (40-100%)**

2407 rabbits clinically examined

30.7% affected by at least one suppurative lesions

- foot lesions (52%)
- mastitis (40%)
- dermatitis/arthritis (17.5%)
- cutaneous abscesses (6%)
- other lesions (5%)



Our study: the results

400 *S. aureus* genotyped



- 5 PFGE clusters (A, B, B1, C, D) ← **D**
- 11 Sequence types (ST: MLST Types) ← **ST121**
- 30 *spa* types



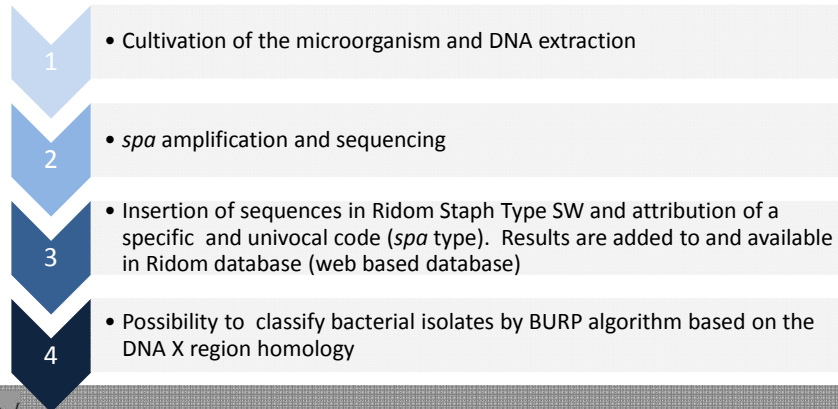
The *spa* typing discriminatory power is higher compared to PFGE and MLST typing



spa typing

Classifies *S. aureus* on the base of X region polymorphism of *spa* gene

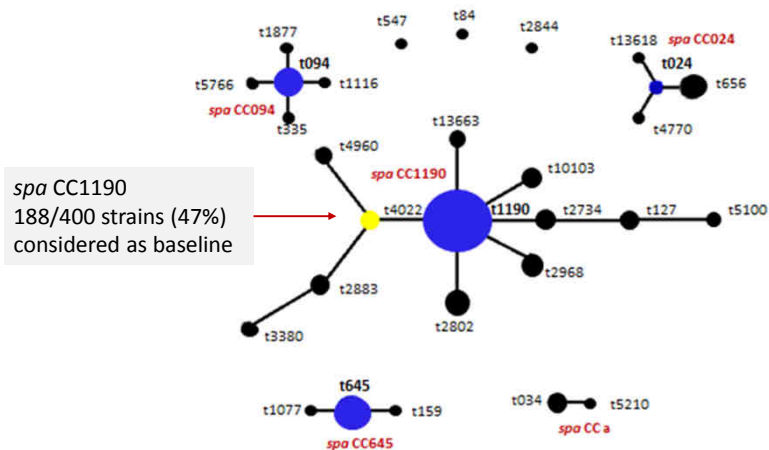
- high discriminatory power
- low cost (only one gene sequencing)
- high inter and intra-laboratories reproducibility
- international standardized nomenclature



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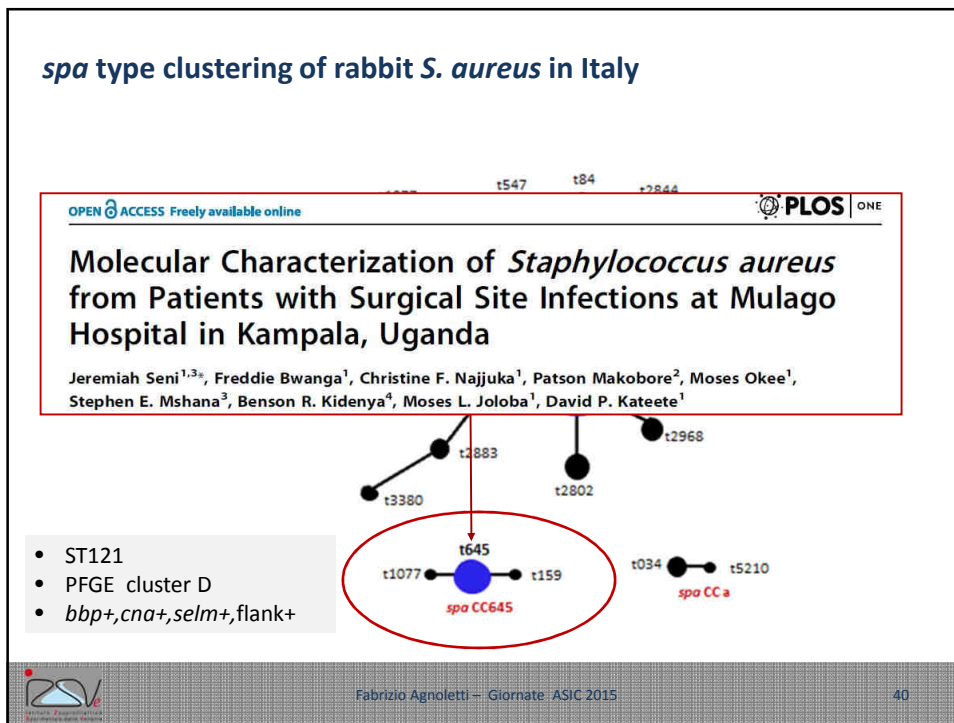
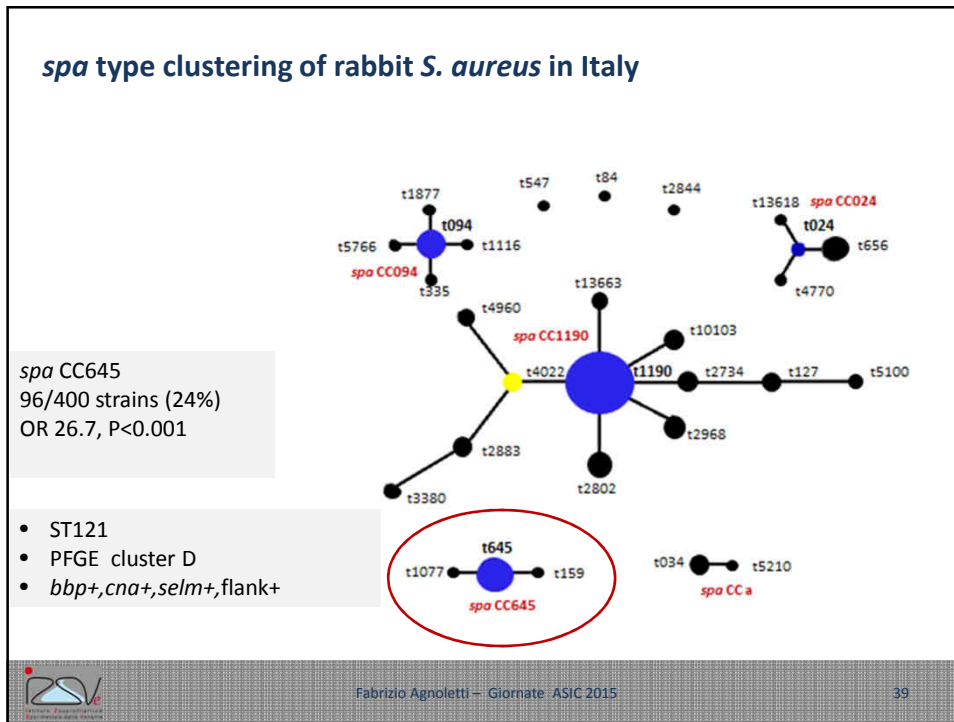
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spa type clustering of rabbit *S. aureus* in Italy



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spa type clustering of rabbit *S. aureus* in Italy

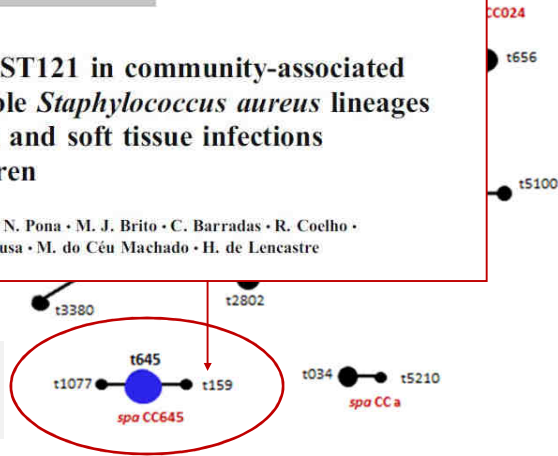
Eur J Clin Microbiol Infect Dis (2011) 30:293–297
 DOI 10.1007/s10096-010-1087-8

BRIEF REPORT

High prevalence of ST121 in community-associated methicillin-susceptible *Staphylococcus aureus* lineages responsible for skin and soft tissue infections in Portuguese children

T. Conceição · M. Aires-de-Sousa · N. Pona · M. J. Brito · C. Barradas · R. Coelho · T. Sardinha · L. Sancho · G. de Sousa · M. do Céu Machado · H. de Lencastre

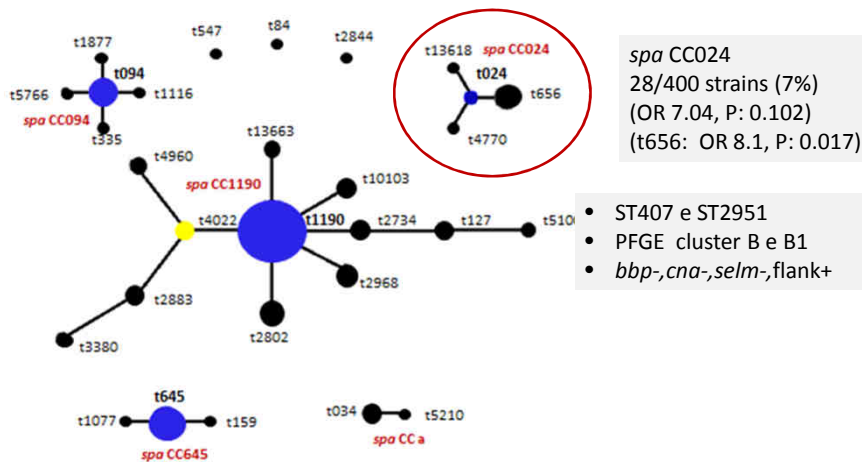
- ST121
- PFGE cluster D
- *bbp+*, *cna+*, *selm+*, *flank+*



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spa type clustering of rabbit *S. aureus* in Italy



spa CC024
 28/400 strains (7%)
 (OR 7.04, P: 0.102)
 (t656: OR 8.1, P: 0.017)

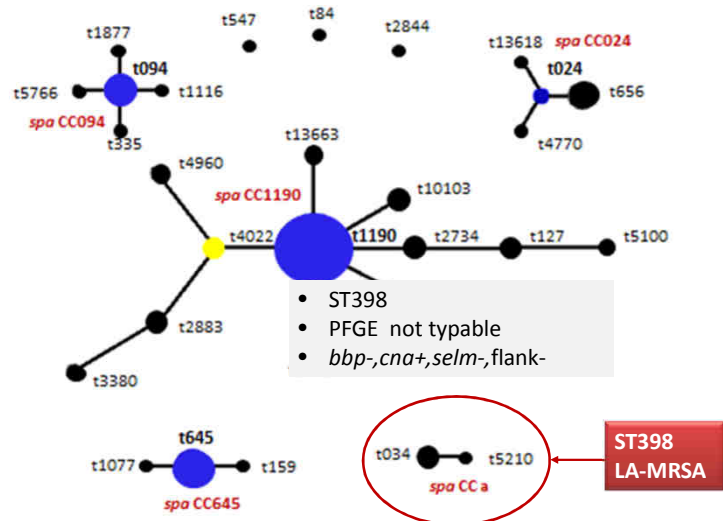
- ST407 e ST2951
- PFGE cluster B e B1
- *bbp-*, *cna-*, *selm-*, *flank+*



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spa type clustering of rabbit *S. aureus* in Italy

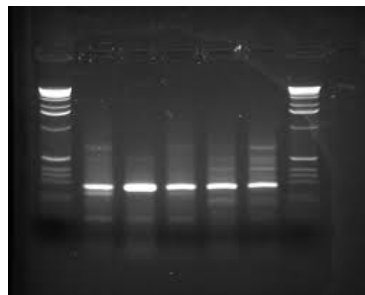


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What's new round the corner on laboratory methods?

The traditional electrophoresis



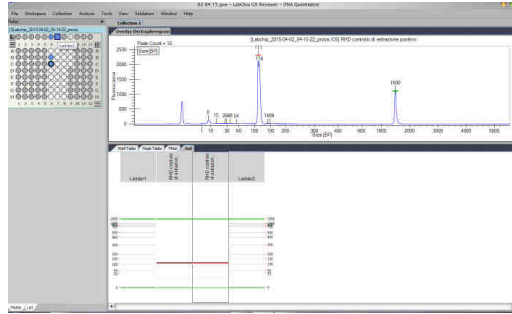
- Gel preparation
- Samples adding
- Electrophoretic run from 1 up to several hours
- Gel staining
- Image acquisition



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What's new round the corner on laboratory methods? The new capillary electrophoresis



- The electrophoretic run occurs within a little chip and needs less than 60 seconds/each sample
- The output is represented by highly portable digital data
- Very high resolution (higher than acrilamide gel)

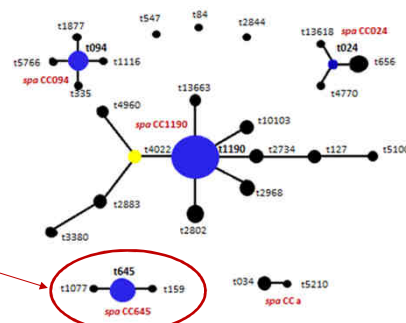


What's new round the corner on laboratory methods?

Multiplex PCR

- *cna*
- *bbp*
- *selm*
- *flank*
- *femA*

based on capillary electrophoresis

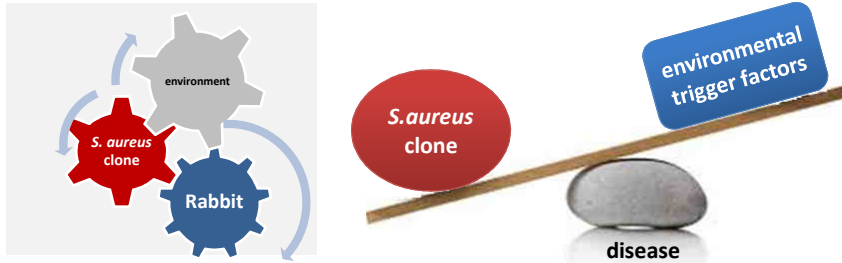


The multiplex PCR for *bbp*, *selm*, *cna* and *flank* is specific for the ST121 clone



Conclusions

The multiplex PCR for *bbp*, *selm*, *cna* and flank sequence detects the ST121 *S. aureus* clone and it is useful when applied to eradicate this specific clone (*spa* CC645) from rabbit herds.



To understand:

- the causes of clinical staphylococcosis in rabbit flocks
 - the role of environmental trigger factors
 - the role of *S. aureus* clone virulence features
- and to have prognostic elements, *spa* typing is a powerful and irreplaceable laboratory tool.



Thanks for your attention!

