éradication du portage de *Staphylococcus aureus.* Pour qui, pourquoi, comment ?

Déterminants

Pr. François Vandenesch

CNR des staphylocoques

INSERM U1111-CNRS UMR5308

Disclosure

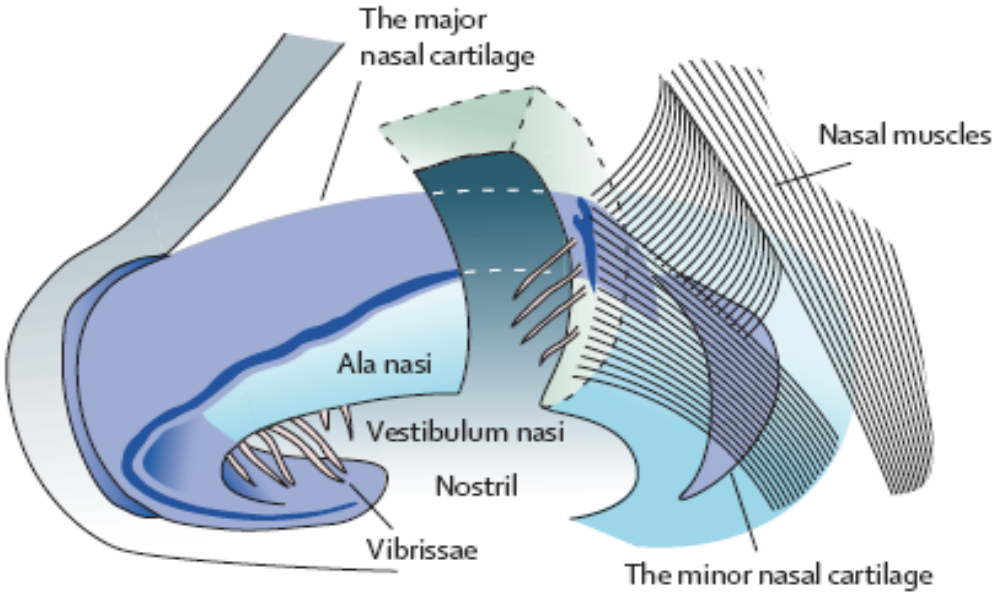
Research Grants, consulting:

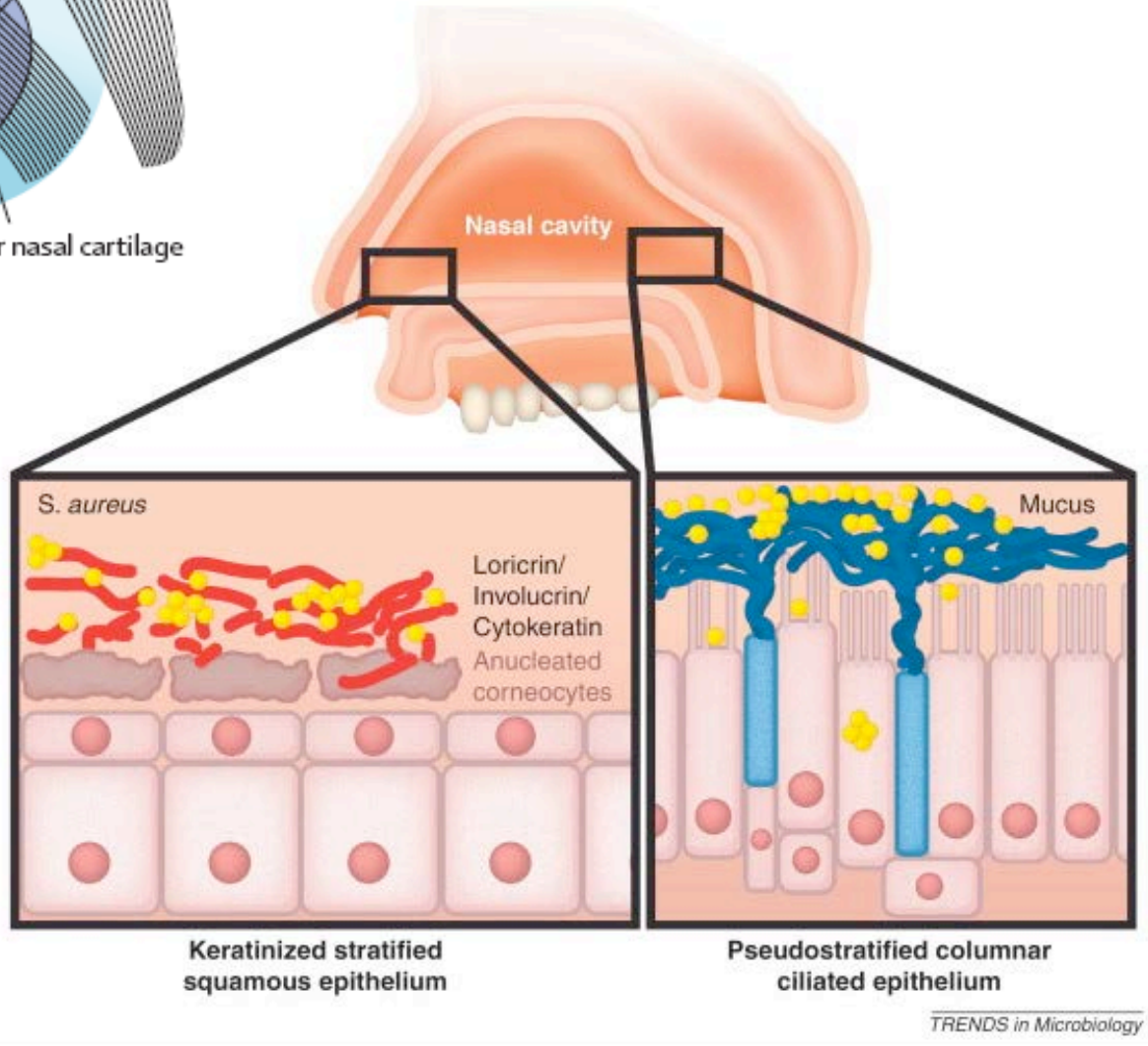
Astra Zeneca, Novartis, Pfizer, bioMérieux, Sanofi-Pasteur

Outlines

- **Definitions**
- **The different categories of carriers**
- **The various sites of carriage**
- **Factor affecting carriage**
- **Interventions : decontamination, vaccination, bacterial interference**

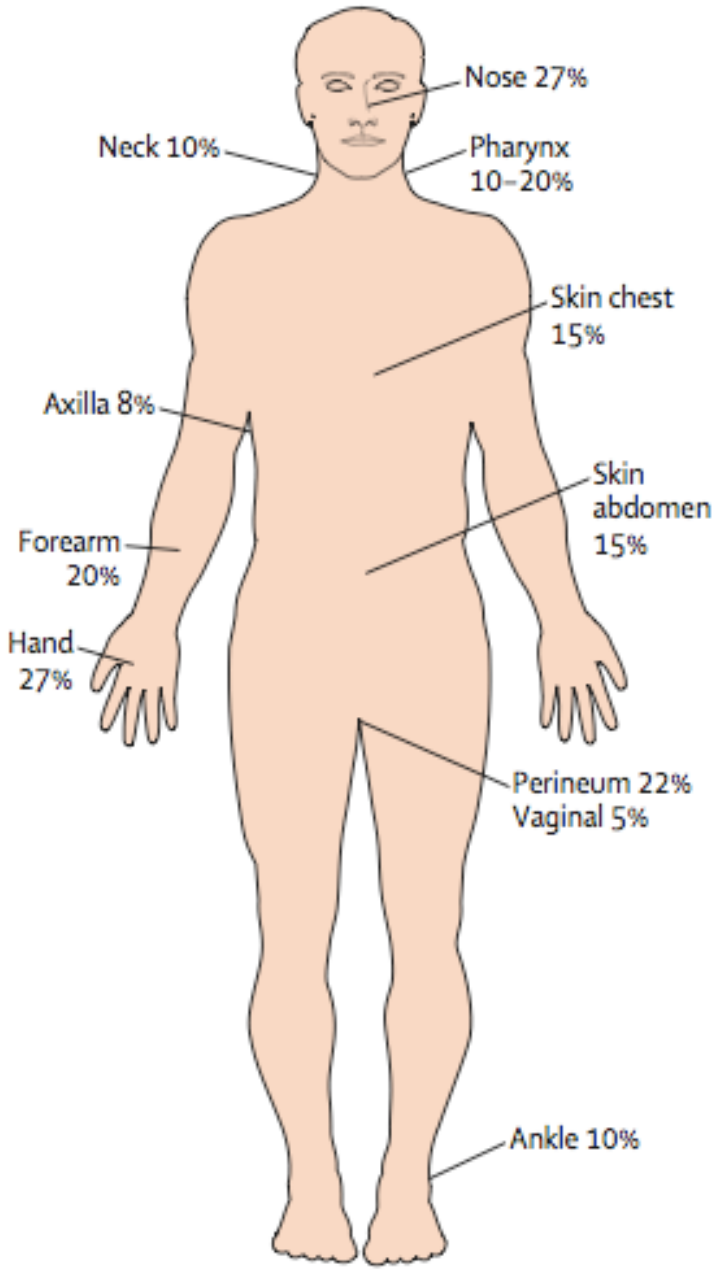
Lancet Infect
Dis2005;5:751-62



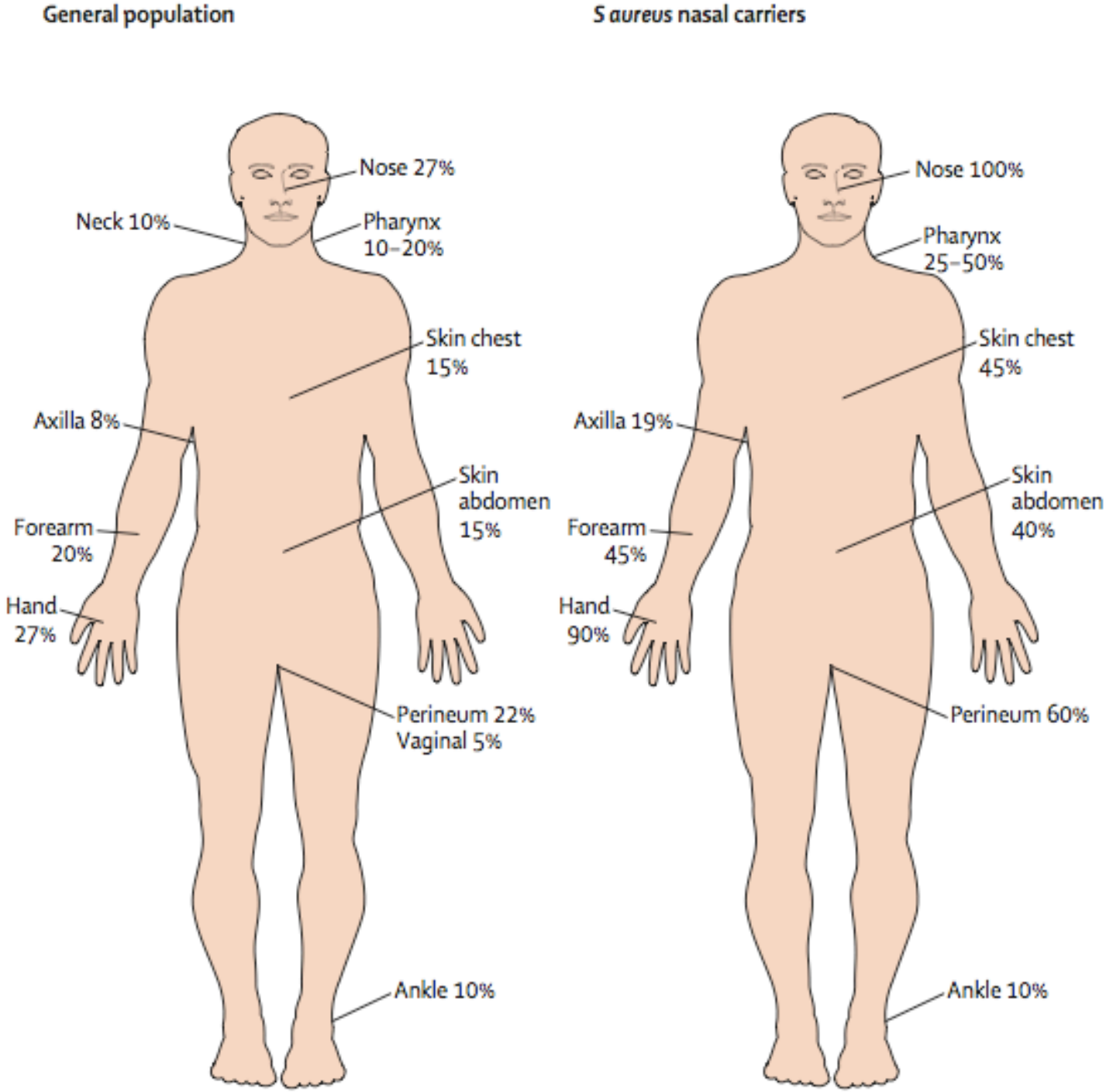


Weidenmaier et al
Trend Microbioly 2012

General population



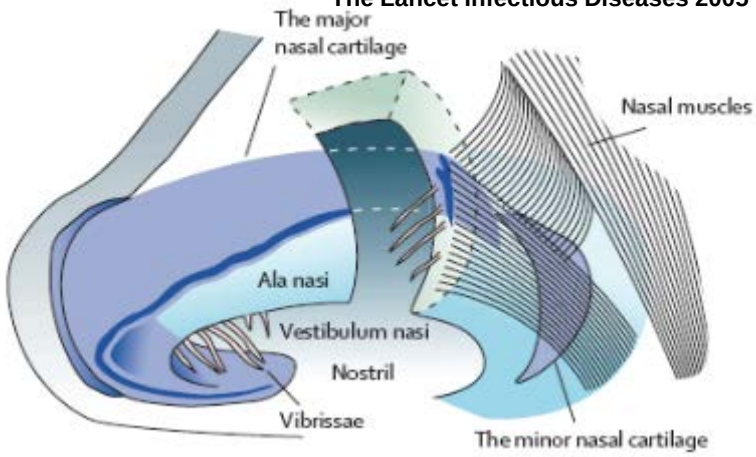
Lancet Infect Dis2005;5:751-62



Lancet Infect Dis2005;5:751-62

nasal carriage of *S. aureus* and the development of staphylococcal infection are linked

Heiman F. L Wertheim et al.
The Lancet Infectious Diseases 2005



- rates of infection are higher in carriers than in non-carriers
- individuals are usually infected with their own carriage isolate
- temporary eradication of carriage following the use of topical mupirocin has been shown to reduce nosocomial infection

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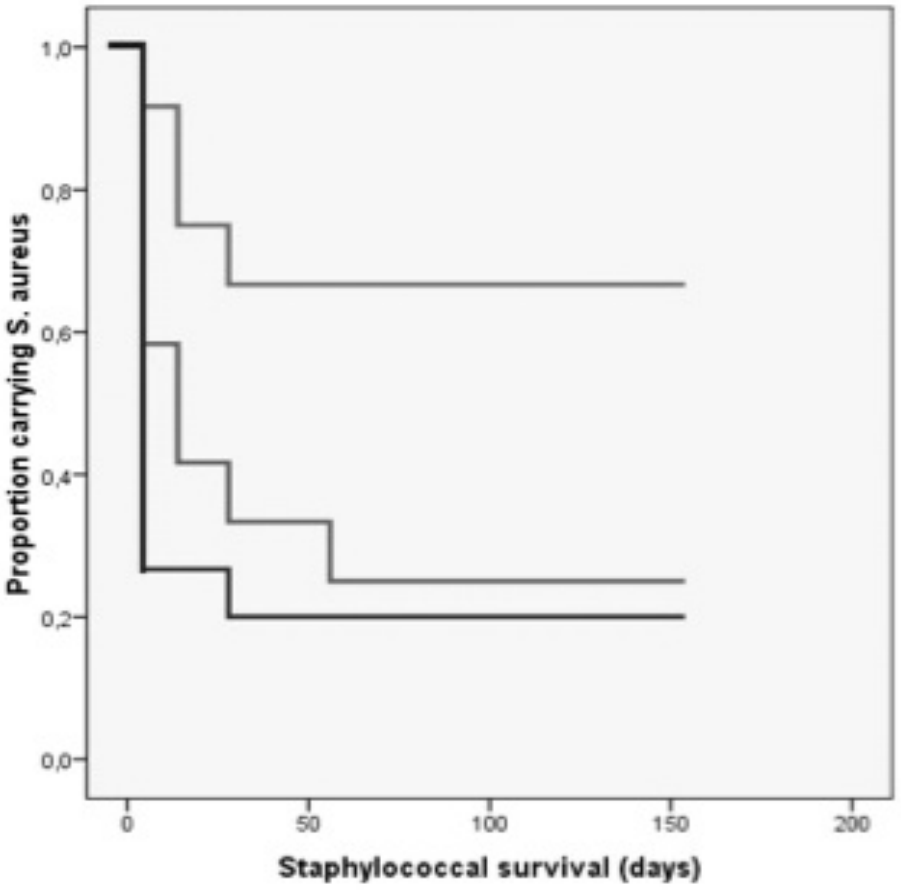
Colonisation study in human



- **Volunteers decolonized and then artificially colonized with a mixture of *S.aureus***
- **Intermitent carriers behave as non carriers:**
  - **Eliminate the inoculum in few days**
- **Persistent carriers preferentially reselected their autologous strain from the inoculum mixture**

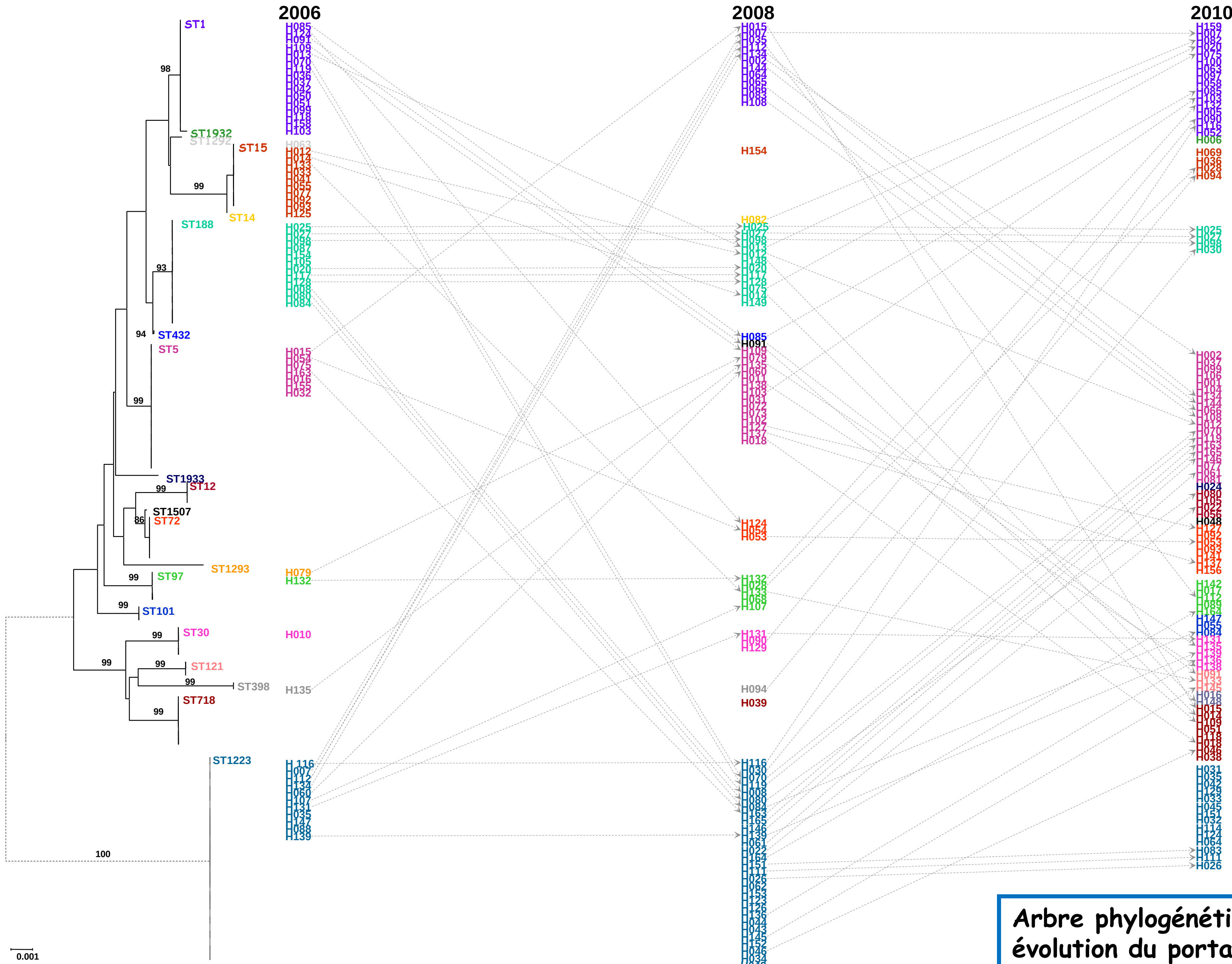
Van Belkum et al.. J INFECT DIS (2009)

The paradigm to be changed

- **Only two categories of people :**
  - **persistent carrier**
  - **non persistent carriers (non carrier and intermittent carrier)**
- **Majority of people are non carriers**

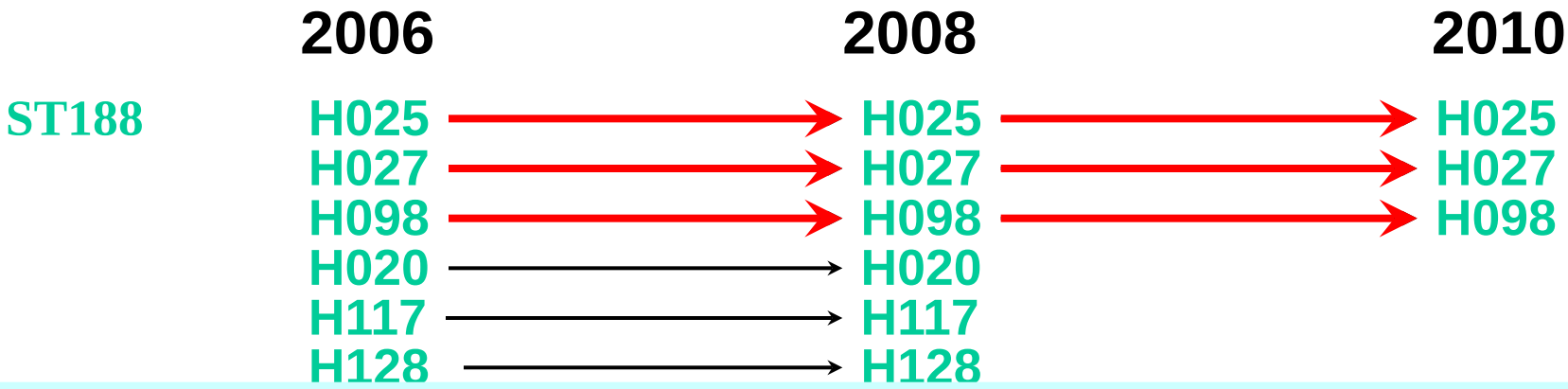
Stability of persistent carrier strains ?

- **Classical model : persistent carrier carry preferentially one strain = well adapted ?**
- **Is it true on the long term?**

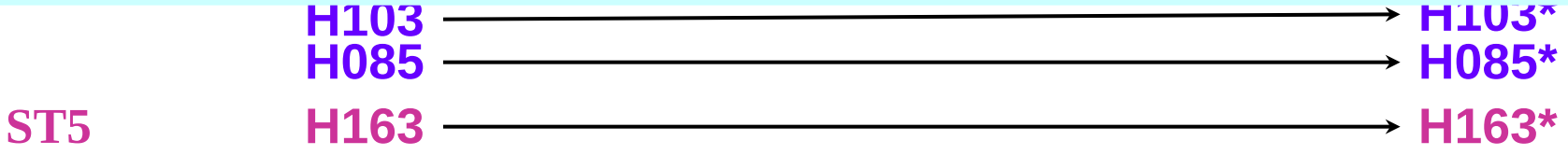


Arbre phylogénétique et évolution du portage

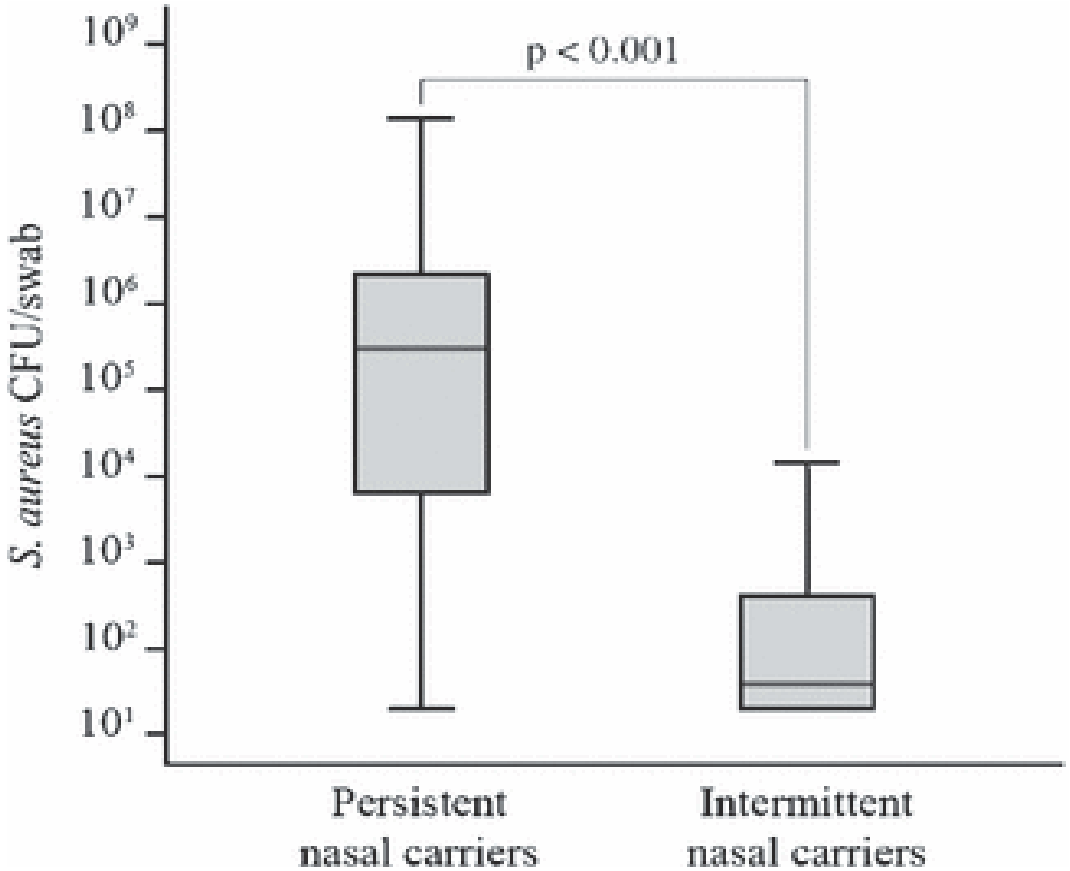
Porteurs de souches de ST identiques



Au total seuls 3 individus portent le même ST (ST188) parmi 29 porteurs permanents



Persistent nasal carriers can be identified on the basis of bacterial load in the nose



Verhoeven et al. An algorithm based on one or two nasal samples is accurate to identify persistent nasal carriers of Staphylococcus aureus
Clinical Microbiology and Infection 2011

Carrier vs autoinfection

- **Persistent carriers are at a higher risk for development of *S. aureus* infections**
- **intermittent carriers have a lower risk of autoinfections than persistent carriers**

vonEiffC,BeckerK,MachkaK,StammerH,PetersG.Nasalcarriageas a source of Staphylococcus aureus bacteremia. N Engl J Med 2001; 344: 11– 6.

Wertheim HF, Vos MC, Ott A, et al. Risk and outcome of nosocomial Staphylococcus aureus bacteraemia in nasal carriers versus non-carriers. Lancet 2004; 364:703–5.

Nouwen JL, Fieren MW, Snijders S, Verbrugh HA, van Belkum A. Per- sistent (not intermittent) nasal carriage of Staphylococcus aureus is the determinant of CPD-related infections. Kidney Int 2005; 67:1084 – 92.

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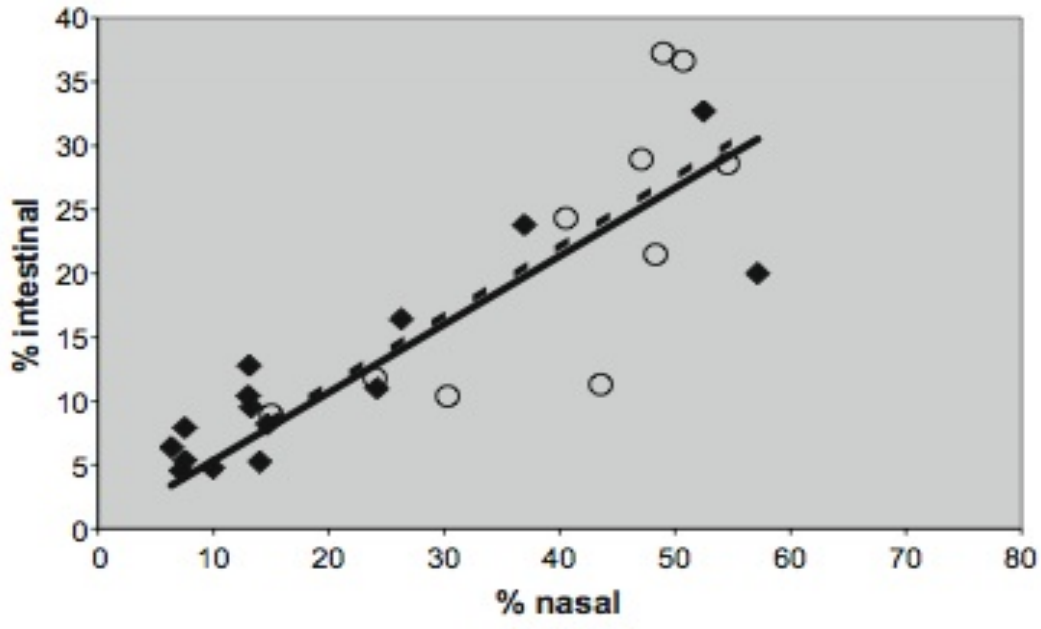
Other sites of carriage : intestine

- **intestinal carriage in healthy individuals and patients is 20% for S. aureus and 9% for MRSA**
- **sole intestinal carriage is observed in 1 out of 3 intestinal carriers**



S. aureus (circles)
MRSA (diamonds)

Acton DS et al. Eur J Clin Microbiol Infect Dis 2009

Other sites of carriage: throat

- **Sole throat carriage observed in 12.8% of 2966 individuals screened for *S. aureus* carriage with swabs of both nares and throat.**

- **independent risk factor for exclusive throat carriage:**
  - **age 30 years or younger (odds ratio, 1.66; p=0.001)**
  - **Protective effect : exposure to the HCS (odds ratio, 0.67; P = 0.001).**
  - **Healthy blood donors were almost twice as likely to have exclusive throat carriage than in-hospital patients and HCWs (30.2% vs 18.4% of all carriers, P=0.001)**

Mertz D et al. Clin Infect Dis 2007
Mertz D et al. Arch Intern Med 2009

Multi sites and CA-MRSA

- Cross-sectional study
- 173 randomly selected sexually transmitted disease clinic clinic
- multiple body site colonization: nare, oropharynx, groin, rectum, and genitals
- USA300 colonized multiple sites more frequently than other strains
- USA300 colonized pelvic sites and the rectum more frequently than non-USA300 strains

Miko et al. High prevalence of colonization with Staphylococcus aureus clone USA300 at multiple body sites among sexually transmitted disease clinic patients: an unrecognized reservoir
Microbes and Infections 2012

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Developped countries have a higher rate of carriage?

- **Europe : 21·6% (n=6956)**
- **Turkey : 10%**
- **Indonesia : <10%**
- **Nigeria : 14%**
- **Mali : 19.6%**

den Heijer CDJ et al, Lancet Infect Dis 2013

Erdenizmenli M et al. Investigation of colonization with methicillin-resistant and methicillin-susceptible staphylococcus aureus in an outpatient population in turkey. Jpn. J. Infect. Dis 2004

Severin JA et al. Unusually high prevalence of panton-valentine leukocidin genes among methicillin-sensitive staphylococcus aureus strains carried in the indonesian population. J. Clin. Microbiol 2008

Adesida SA et al. Associated risk factors and pulsed field gel electrophoresis of nasal isolates of staphylococcus aureus from medical students in a tertiary hospital in lagos, nigeria. Braz. J. Infect. Dis 2007

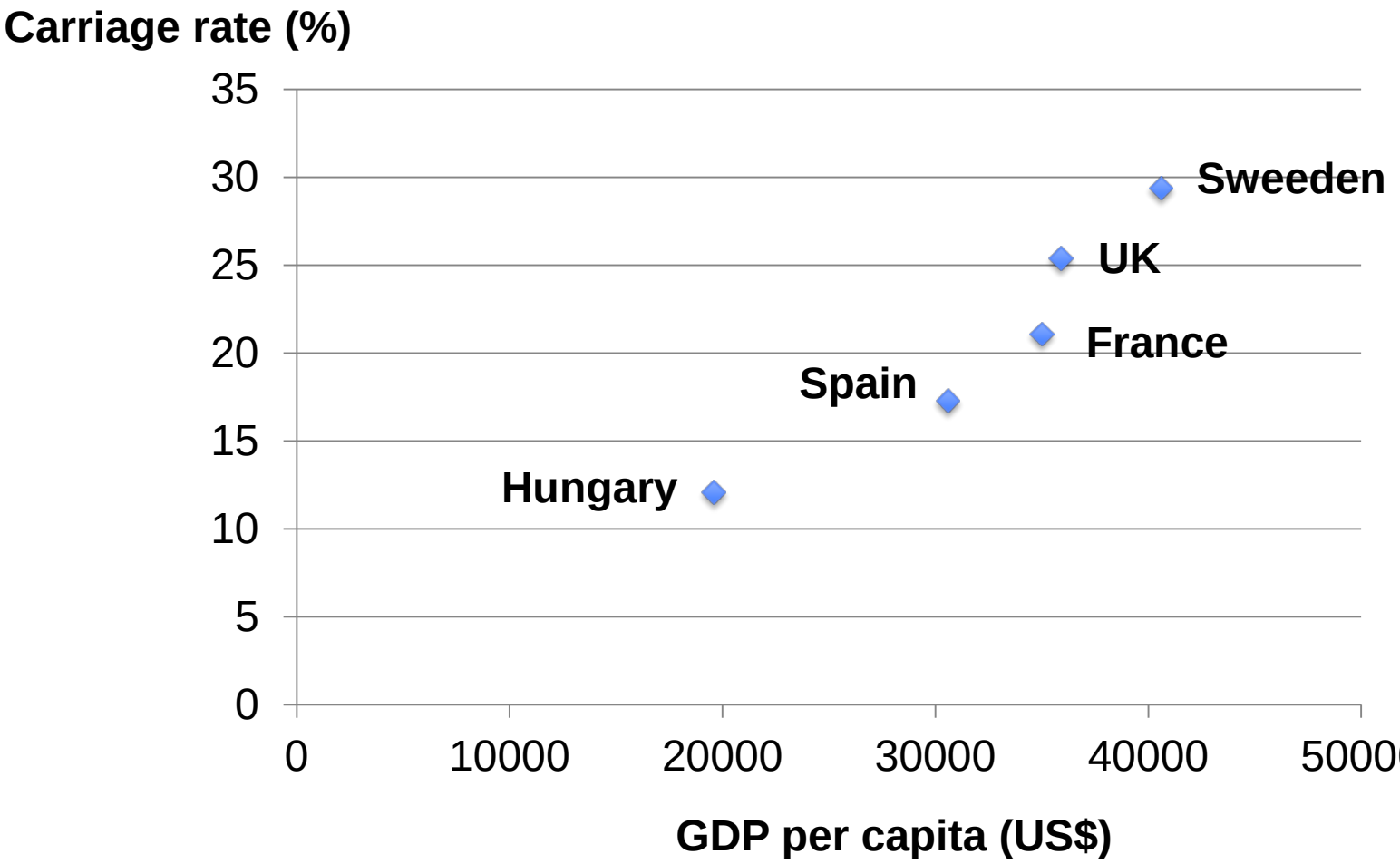
Ruimy R et al The carriage population of *Staphylococcus aureus* from Mali is composed of a 2 combination of pandemic clones and the divergent PVL positive genotype ST152. J.Bacteriol 2008

Variations between Countries with similar access to health and hygiene

- **32 206 patients / 9 European countries through a family doctor network.**
- **Overall *S.aureus* carriage: 21·6% (n=6956)**
  - **12.1% in Hungary**
  - **17.3% in Spain**
  - **21.1% in France**
  - **25.4% in UK**
  - **29·4% in Sweden**

den Heijer CDJ et al, Lancet Infect Dis 2013

Carriage in Europe vs GDP per capita !



Carriage data from den Heijer CDJ et al, Lancet Infect Dis 2013

Carriage may result of both Ethnicity and availability of health and hygiene

- **Europe : 21·6% (n=6956)**
- **Turkey : 10%**
- **Indonesia : <11%**
- **Nigeria : 14%**
- **Mali : 19.6%**
- **Wakampi amerindians : 56%**

Ruimy R et al. Are Host Genetics the Predominant Determinant of Persistent Nasal Staphylococcus aureus Carriage in Humans? J Infect Dis 2010

23rd ECCMID

Berlin, Germany
27–30 April 2013

Basic rules of hygiene protect health care and lab workers from nasal colonization by *Staphylococcus aureus*: an International Cross-Sectional Study

*M. Saadatian-Elahi, A. Tristan, F. Laurent, JP. Rasigade, C. Bouchiat, AG. Ranc, G. Lina, O. Dauwalder, J. Etienne, M. Bes, F. Vandenesch

Lyon 1

Inserm
Institut national
de la santé et de la recherche médicale

CIRI
Centre International de
Recherche en Infectiologie

15th ISSSI LYON 26-30 AUG 2012

Hôpitaux de Lyon

STAPHYLOCOQUES
Centre National de Référence

Subjects exposed to the pathogen in their working environment

- **123 subjects worked in laboratories with direct manipulation of *S. aureus* on a regular basis (1-3 times per week)**

- **29 subjects had regular contact with patients**

<u>Compliance with good laboratory practices</u>

- **Use of gloves, long sleeves and hydro-alcoholic solutions: 75%**
- **Dedicated shoes: 13%**
- **Safety cabinet: 52%**

Factors associated with the risk of nasal *S. aureus* carriage in the exposed population

Univariate analysis

Variable	Crude OR (95% CI)	p-value
Use of safety cabinet (laboratory workers)	0.86 (0.41-1.81)	0.69
Use of gloves	1.32 (0.55-3.17)	0.53
Use of long sleeves	0.72 (0.32-1.61)	0.42
Use of dedicated shoes	0.39 (0.11-1.44)	0.16
Use of hydro-alcoholic solutions	**0.42 (0.19-0.96)**	**0.04**
Direct manipulation of *S. aureus*	0.85 (0.43-1.69)	0.64
Regular contact with patients	1.28 (0.54-2.99)	0.58
Working in hospital & lab	1.65 (0.86-3.11)	0.13

Use of hydro-alcoholic solutions (HAS), is associated with decreased risk of nasal *S. aureus* colonization

Multivariate analysis

Variable	Adjusted OR (95% CI)	p-value
Use of hydro-alcoholic solutions	0.36 (0.15-0.85)	0.02
Working in hospital & lab	2.38 (1.07-5.29)	0.03

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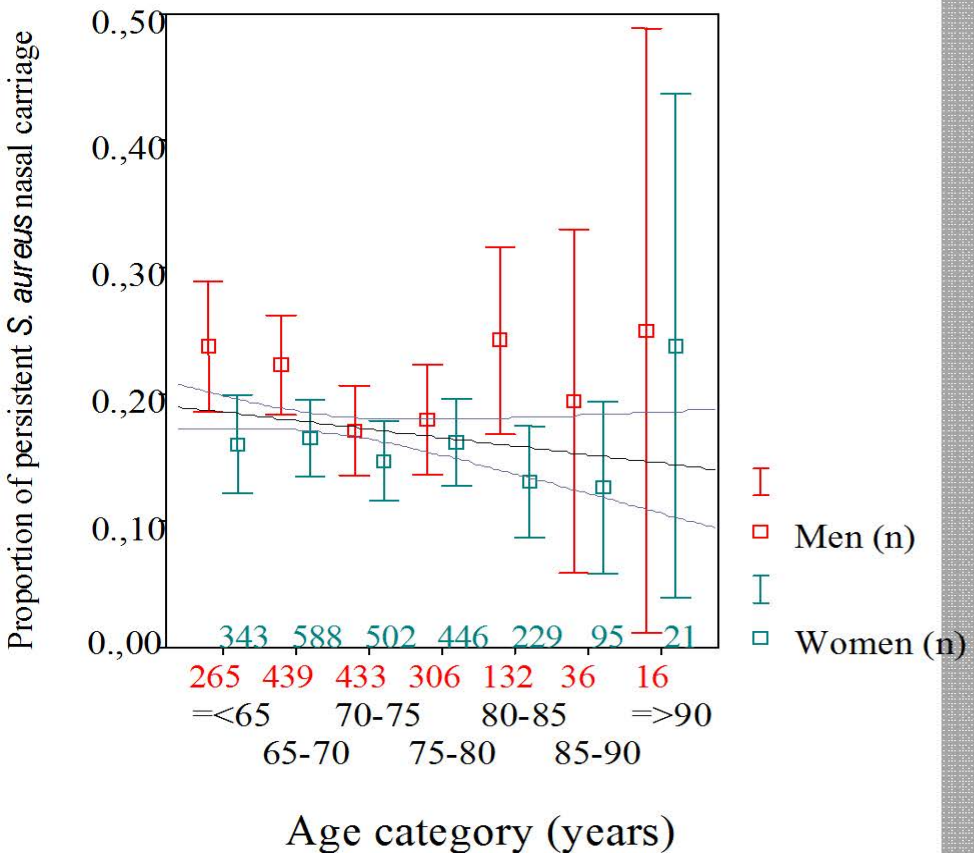
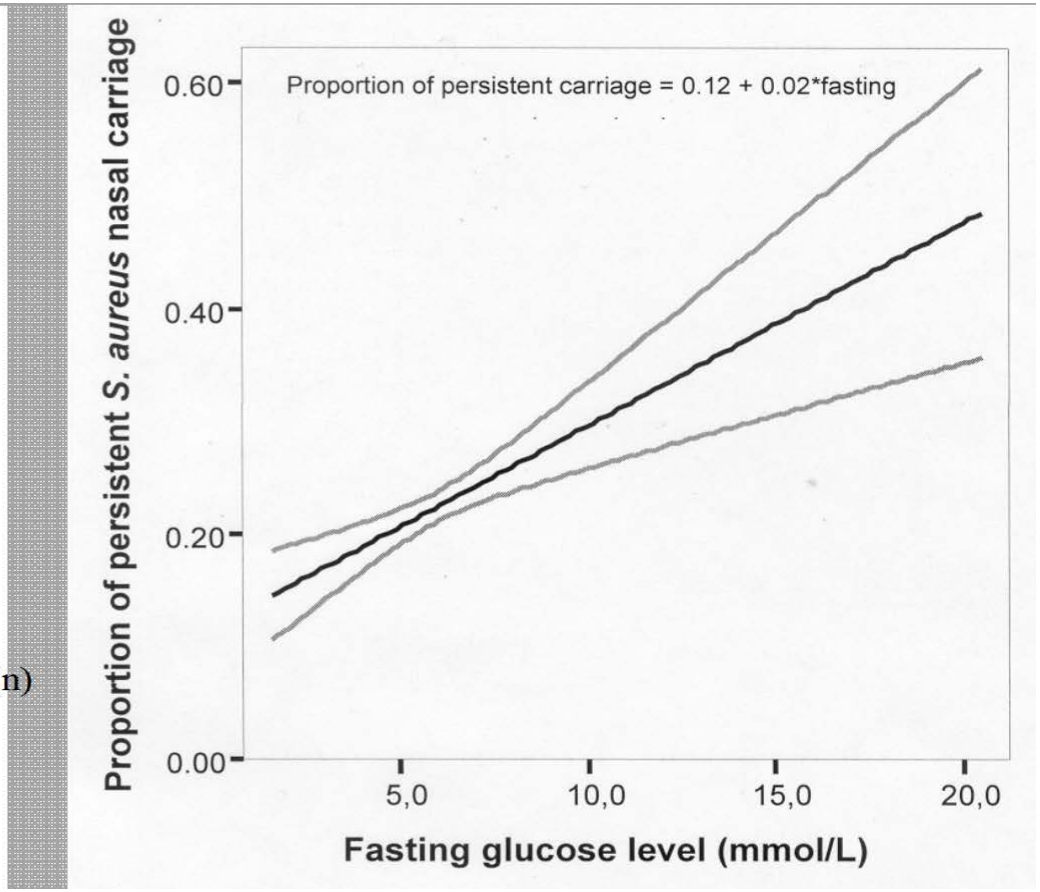
Host : age, gender and glucose level

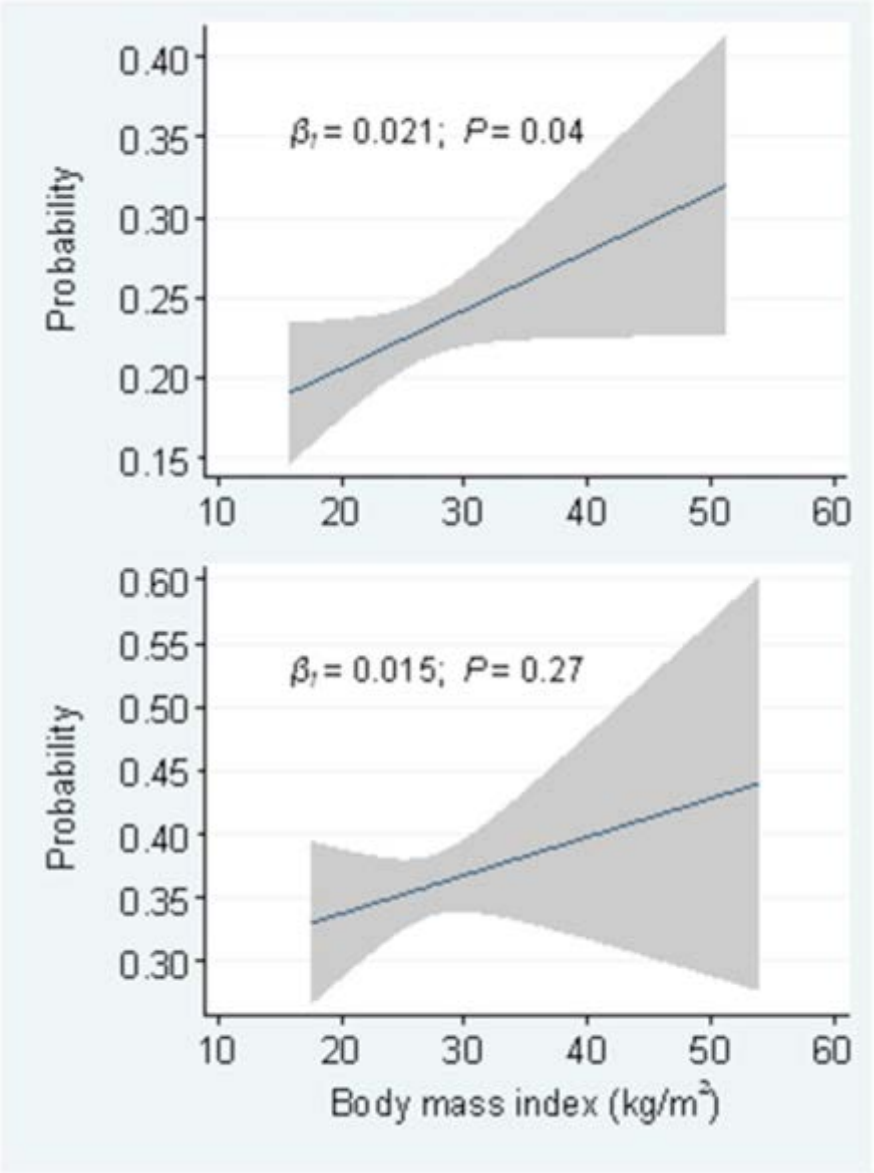




Nouwen J et al (2004) PhD thesis. Erasmus University Medical Centre, Rotterdam, The Netherlands pp. 17–3

Body mass

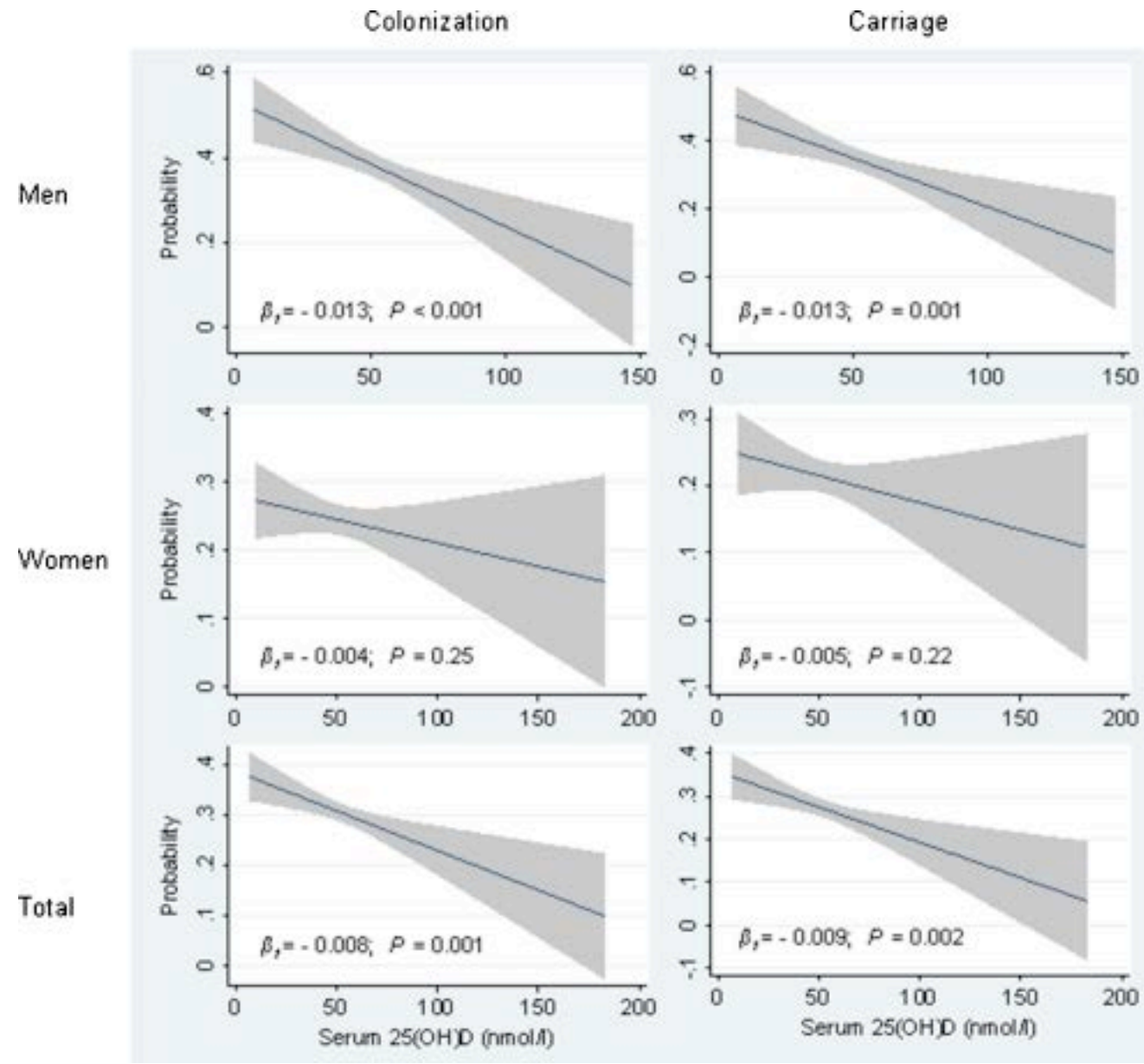
- **For each 2.5 kg/m2 increase in BMI a 7% increase in the odds of *S. aureus* nasal colonization**
- **A risk factor for carriage independ of Diabetes Melitus**



Olsen et al, Obesity and Staphylococcus aureus Nasal Colonization among Women and Men in a General Population. PLOS ONE 2013

Vitamin D level

- **Vitamin D induces the expression of antimicrobial peptides**



Olsen et al. Staphylococcus aureus nasal carriage is associated with serum 25-hydroxyvitamin D levels, gender and smoking status. The Tromsø Staph and Skin Study
Eur J Clin Microbiol Infect Dis 2012

Variation in Nasal secretion

- **Nasal fluid = numerous antimicrobial proteins and peptides such as:**
  - **lysozyme,**
  - **lactoferrin,**
  - **Secretory Leukocyte Protease Inhibitor (SLPI),**
  - **neutrophil alpha-defensins,**
  - **beta-defensins and cathelicidins**
- **HBD3, cathelicidin (LL-37), and lysozyme are synergistic against S.aureus**
- **Dysregulation of expression of one or more of these innate peptides might predispose a person to carriage**
- **HBD3 level is lower in persistent carriers vs non-carriers**

Chen X et al. Synergistic effect of antibacterial agents human beta-defensins, cathelicidin LL-37 and lysozyme against staphylococcus aureus and escherichia coli. J. Dermatol. Sci 2005

Sivaraman et al. *Staphylococcus aureus* Nasal Carriage and its Contributing Factors. Future Microbiol 2009

Nurjadi et al,Impaired β-defensin expression in human skin links DEFB1 promoter polymorphisms with persistent Staphylococcus aureus nasal carriage. J. Infect Dis 2013

Predisposing Host Genetic factors

- **Presence of HLA-DR3**
- **Polymorphic variations of numerous genes such as :**
  - **Fc fragment of IgG (Fc©R),**
  - **human glucocorticoid receptor,**
  - **vitamin D responsive element (VDREs) in patients with type I diabetes**
  - **IL-4 (C542T genotype)**
  - **CRP (CRP C2042T and C1184T)**
  - **human complement cascade activator serine protease C1 inhibitor (C1INH V480M)**
  - **DEFB1 promoter polymorphisms-> impaired β-defensin expression**

Kinsman OS et al. Association between histocompatability antigens (HLA) and nasal carriage of staphylococcus aureus. J. Med. Microbiol 1983

Panierakis C et al. Staphylococcus aureus nasal carriage might be associated with vitamin D receptor polymorphisms in type 1 diabetes. Int. J. Infect. Dis. 2009

Emonts M et al. Host polymorphisms in interleukin 4, complement factor H, and c-reactive protein associated with nasal carriage of staphylococcus aureus and occurrence of boils. J. Infect. Dis 2008

van den Akker EL et al. Staphylococcus aureus nasal carriage is associated with glucocorticoid receptor gene polymorphisms. J. Infect. Dis 2006

Nurjadi et al,Impaired β-defensin expression in human skin links DEFB1 promoter polymorphisms with persistent Staphylococcus aureus nasal carriage. J. Infect Dis 2013

Table 5. Host Genotype in *Staphylococcus aureus* Persistent Nasal Carriers versus the Other Volunteers

Gene (SNP), allele	No. (%) of persons: *S. aureus* persistent carriers (n = 40)	No. (%) of persons: Other volunteers (n = 114)	OR (95% CI)	P	Global P[a]
IL4 (−524)				.20	.02
C/C	2 (5.3)	1 (0.9)	1.0		
T/C	11 (28.9)	25 (22.9)	0.2 (0.0–4.8)		
T/T	25 (65.8)	83 (76.1)	0.2 (0.0–3.1)		
CRP (1184)				.12	
C/C	24 (61.5)	83 (73.5)	1.0		
T/C	14 (35.9)	23 (20.4)	2.1 (0.9–5.0)		
T/T	1 (2.6)	7 (6.2)	0.5 (0.0–4.2)		
CRP (2042)				.02	
C/C	13 (32.5)	58 (52.3)	1.0		
T/C	23 (57.5)	36 (32.4)	2.8 (1.2–6.9)		
T/T	4 (10.0)	17 (15.3)	1.0 (0.2–4.0)		

NOTE. CI, confidence interval; CRP, C-reactive protein; IL4, interleukin 4; OR, odds ratio; SNP, single nucleotide polymorphism.

[a] P values for each SNP were combined in a global test by Fisher's combination of probabilities test.

- **SNP distribution in CRP C2042T and C1184T and interleukin-4 C524T genes was significantly associated with carriage**

Ruimy et al. Are Host Genetics the Predominant Determinant of Persistent Nasal Staphylococcus aureus Carriage in Humans? J Infect Dis. 2010

Host Genetic factors

- **Glucocorticoid Receptor Gene Polymorphisms**
  - **2929 subject enrolled**
  - **GG homozygotes of the exon 9beta polymorphism were associated with a 68% reduced risk of persistent *S. aureus* nasal carriage,**
  - **Presence of the codon 23 lysine allele displayed an 80% increased risk**
  - **Hypothesis: cortisol resistance (lysine allele) leads to elevated levels of cortisol induces immune suppresssion and increases the risk of being a persistent carrier**

van den Akker et al. J Infect Dis (2006) vol. 194 (6) pp. 814-8

Host Genetic factors identified in mice or cellular models

- **CFTR and TLR-2 protect against nasal colonization / carriage**
- **A carrier clinical isolate was capable of suppressing TLR expression in human nasal epithelial cells**

González-Zorn Bet al. Bacterial and host factors implicated in nasal carriage of methicillin-resistant staphylococcus aureus in mice. Infect. Immun 2005

Quinn GA et al. Suppression of innate immunity by a nasal carriage strain of staphylococcus aureus increases its colonization on nasal epithelium. Immunology 2007

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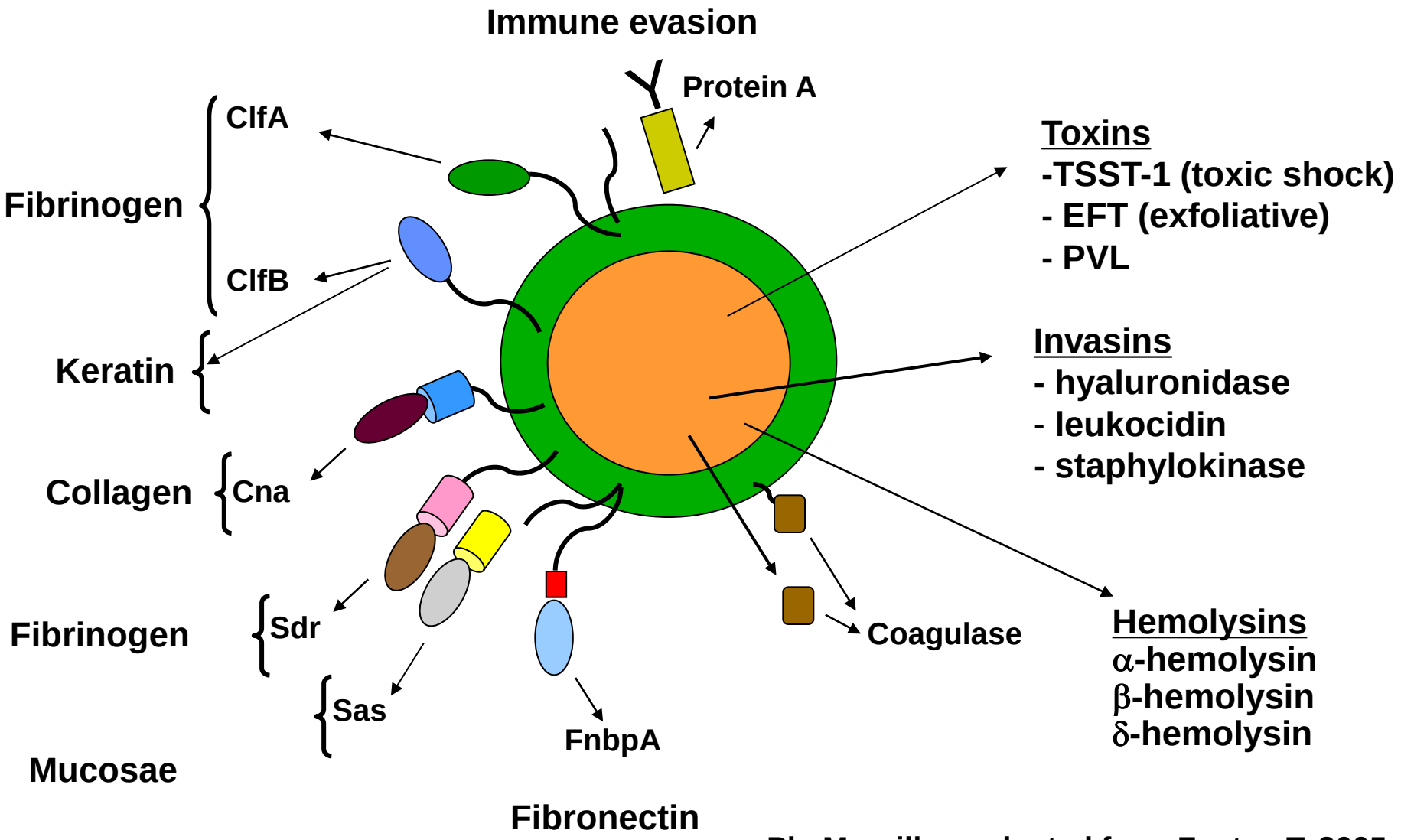
The Bug

- **Core genome**
- **Core variable: factors with genetic variability**
- **Variable genome: includes PI**

Virulence Factors of Staphylococcus aureus : simplified version

Cell-wall Adhesins

Secreted



Ph. Moreillon, adapted from Foster, T. 2005

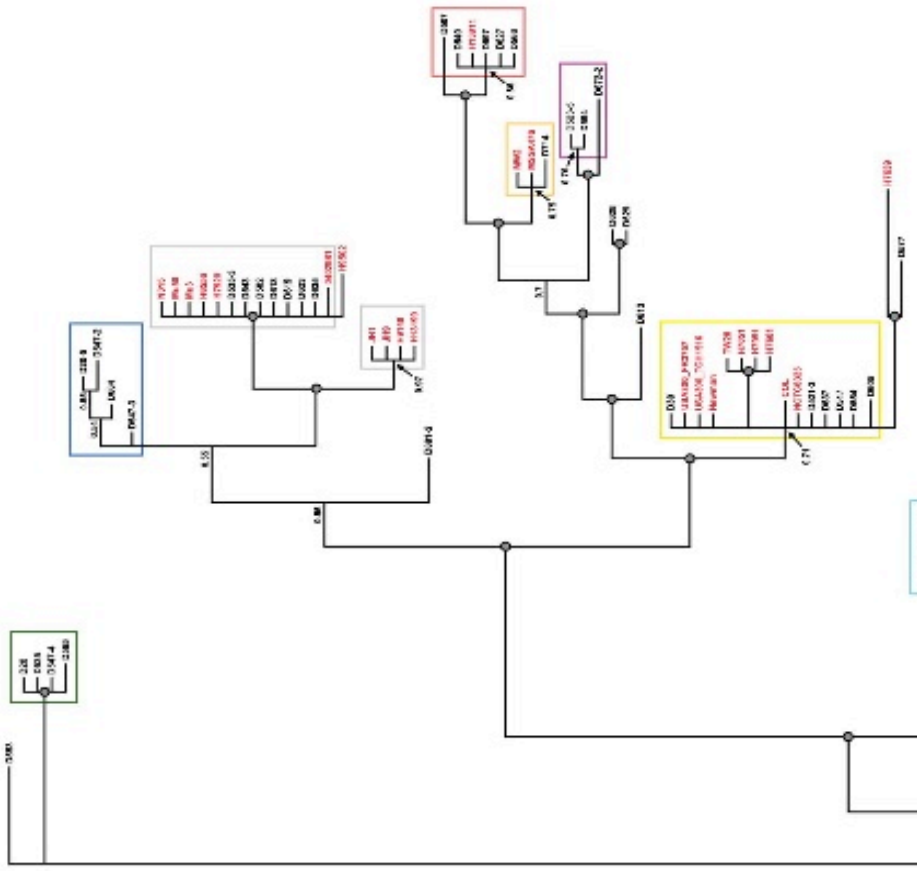
The Bug

- **Core genome**
- **Core variable: factors with genetic variability**
- **Variable genome: includes PI**

- **Questions to address:**
  - **Specific lineages associated with carriage vs disease ?**
  - **Virulence factors responsible for carriage ?**
  - **Distribution of the above factors in carriage vs disease isolates**
- **→ invasive isolates # colonizing isolates?**

The Bug: Lineage or not ?

MLST analysis reveals phylogenetic relationships between *S. aureus* nasal carriage and clinical strains

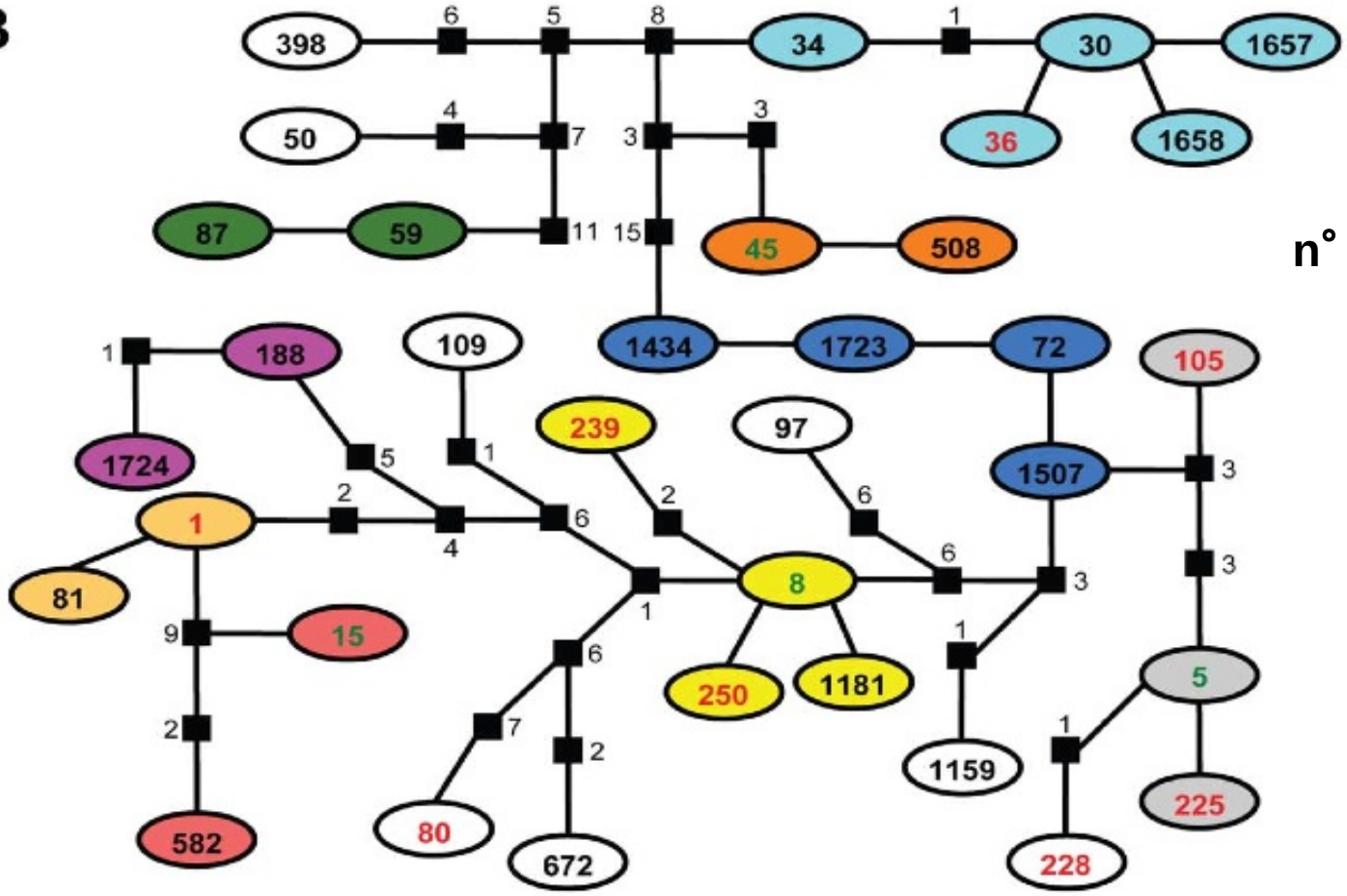


carriage strains in black
clinical strains in red

the vast majority of clade
containing clinical isolate
also contain nasal
carriage

Lamers et al PLOS One. 2011 | Volume 6 | Issue 1 | e16426

Nasal carriage and clinical isolates of *S. aureus* belong to the same genetic clusters



n° in black:carriage strains
n° in red: clinical strains
n° in green: clinical & carriage

Lamers et al PLOS One. 2011 | Volume 6 | Issue 1 | e16426

The Bug: virulence factors for carriage

- **Numerous genes have been shown to play a role**

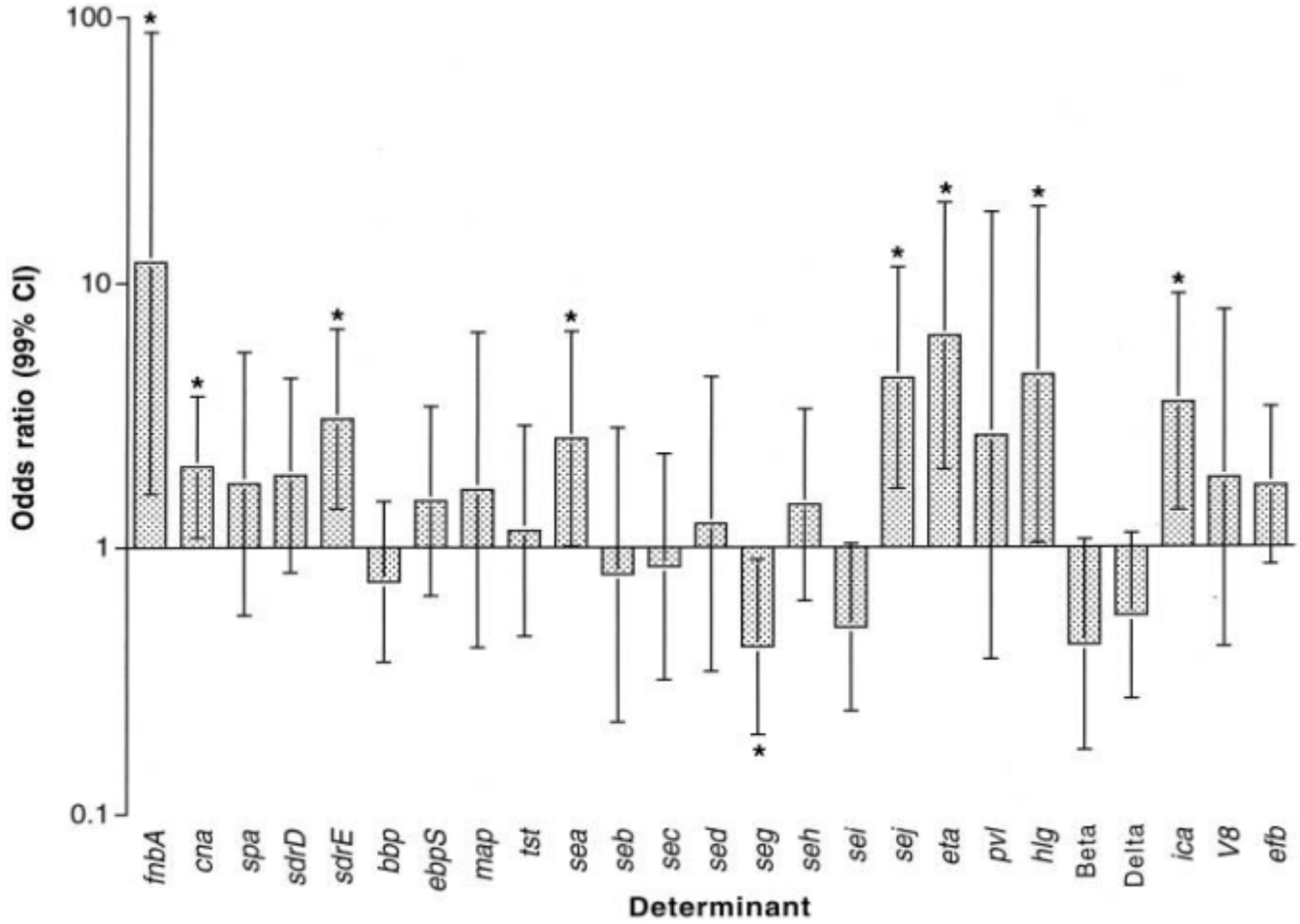
Gene	Remarks
Clumping factor B	adheres to cytokeratin K10 and K8
sdrC, D, E, SasG	Adhesion to desquamated nasal epith cells
fnbA	Fibronectin binding protein
Egc SE	Superantigens
isdA	Siderophore, interacts with the corneocytes enveloppes proteins Locrcirin and Involucrin
SaPIBov	Bovine Pathogencity island
Type 7SS	TraG / FtsK complex
WTA	Adherence to non squamous cells

----→ Specificity for carriage ?

ADAPTED from Sivaraman et al. *Staphylococcus aureus* Nasal Carriage and its Contributing Factors. Future Microbiol 2009

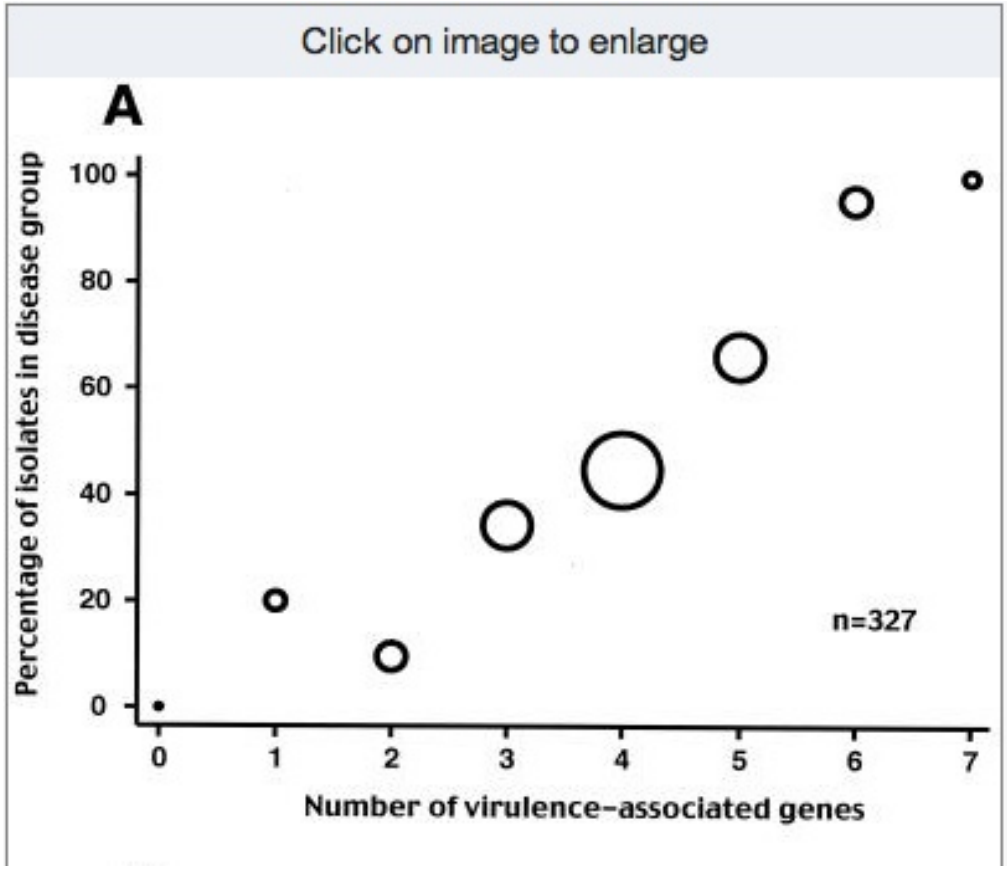
Johannessen et al, Host- and microbe determinants that may influence the success of S. aureus colonization . Frontiers in Cell and Infect Microbiol 2012

Somes virulence determinants are positively associated with disease vs colonization across multiple *S. aureus* lineages



Peacock SJ et al. Virulent combinations of adhesin and toxin genes in natural populations of Staphylococcus aureus. Infect Immun. 2002 Sep;70(9):4987-96

Cumulative effect of virulence genes for disease vs colonization

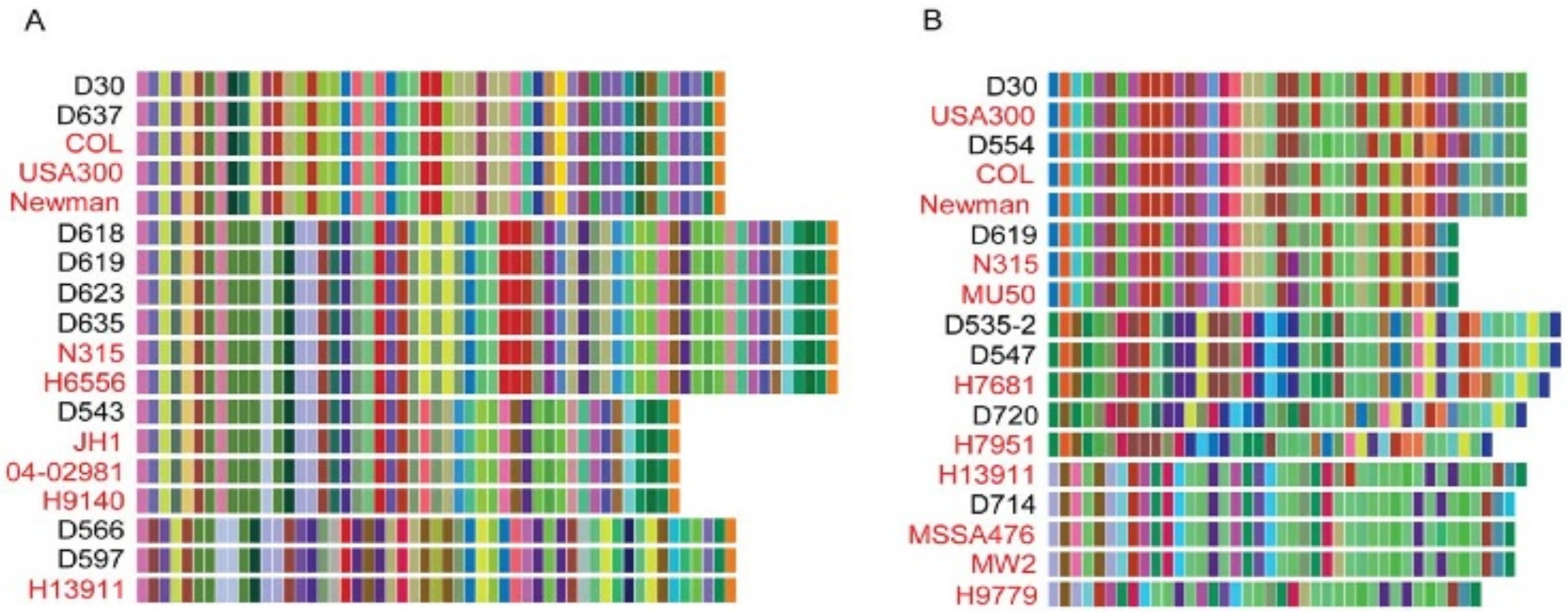


Peacock SJ et al. Virulent combinations of adhesin and toxin genes in natural populations of Staphylococcus aureus. Infect Immun. 2002 Sep;70(9):4987-96

Sequence variability in virulence genes may differentiate colonizing vs disease isolates ?

- **Clf and Fnb are putative determinants of nasal carriage**
- **Length differences within the repeat regions of the clf and fnb genes did not discriminate between colonizing and disease isolates**

Nasal carriage and clinical isolates of *S. aureus* share (near-)identical clf repeat region sequences

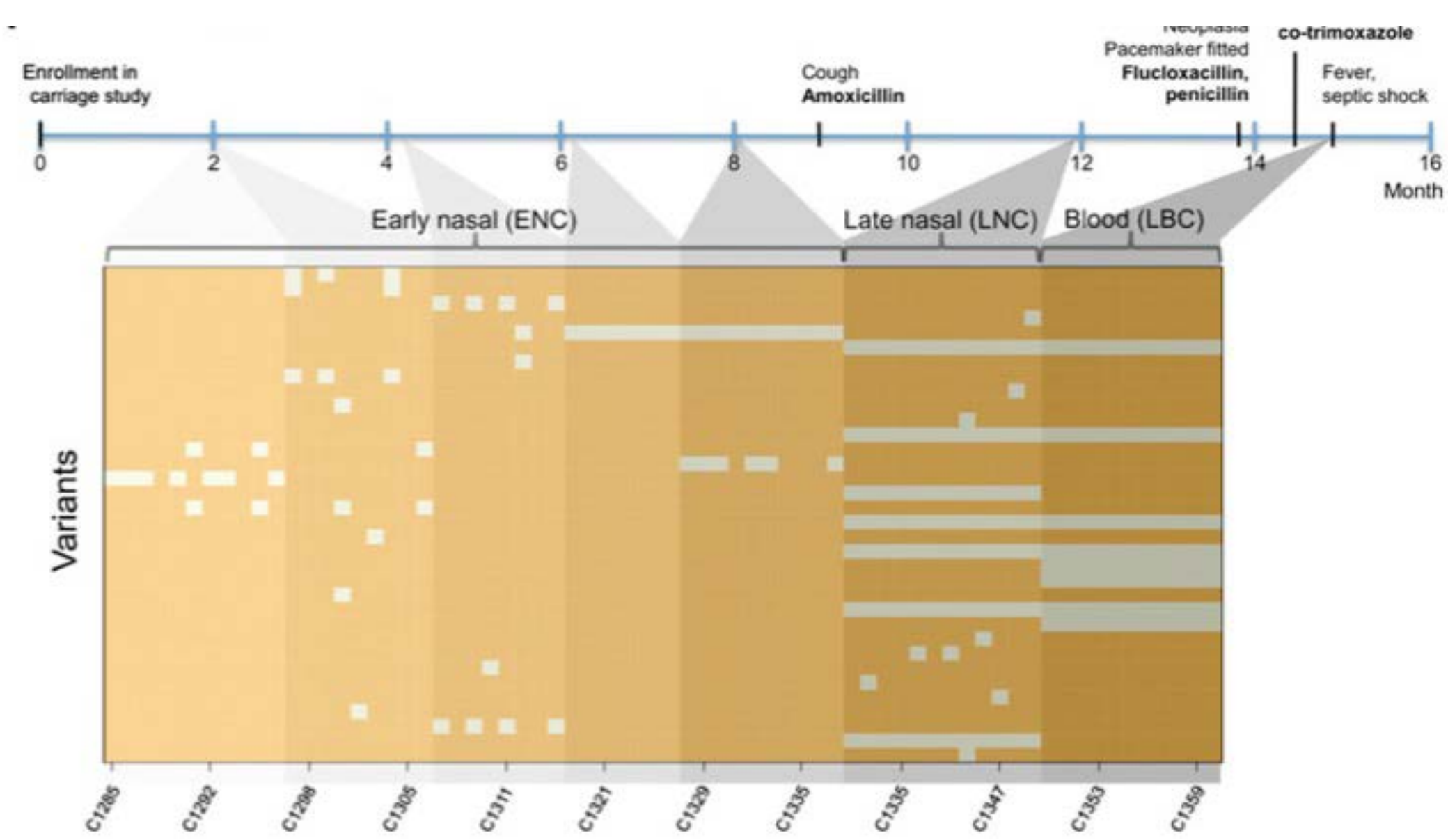


n° in black:carriage strain
n° in red: clinical strains

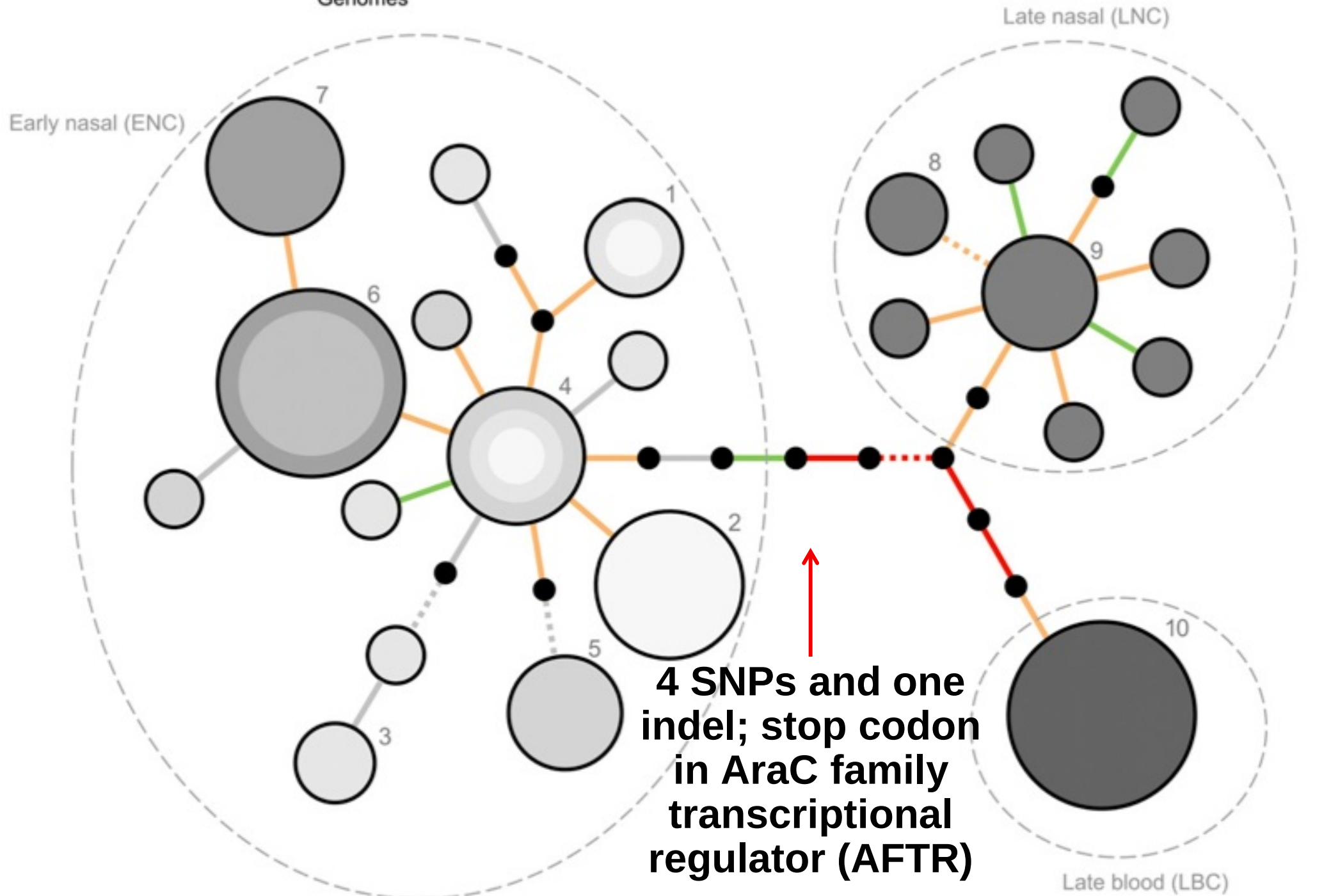
Lamers et al PLOS One. 2011 | Volume 6 | Issue 1 | e16426

The Bug: subtle genomic variations

- **Whole genome sequence of carriage and disease isolates from the same patient**



Young et al, Evolutionary dynamics of Staphylococcus aureus during progression from carriage to disease. PNAS 2012



Young et al, Evolutionary dynamics of Staphylococcus aureus during progression from carriage to disease. PNAS 2012

The bug

- **Nasal carriage and clinical isolates of *S. aureus* belong to the same genetic clusters**
- **A number of virulence determinants are involved in colonization but none are specifically present in colonizing vs disease (except one paper…)**
- **Limited evidence suggesting that colonizing isolates behave differently from disease isolates**

The other Bugs

- **The nasal microbiota**
- **Bacterial competition between *Staphylococcus aureus* and other nares-associated microbial species**
- **Possible way of intervention**

Bacterial interference

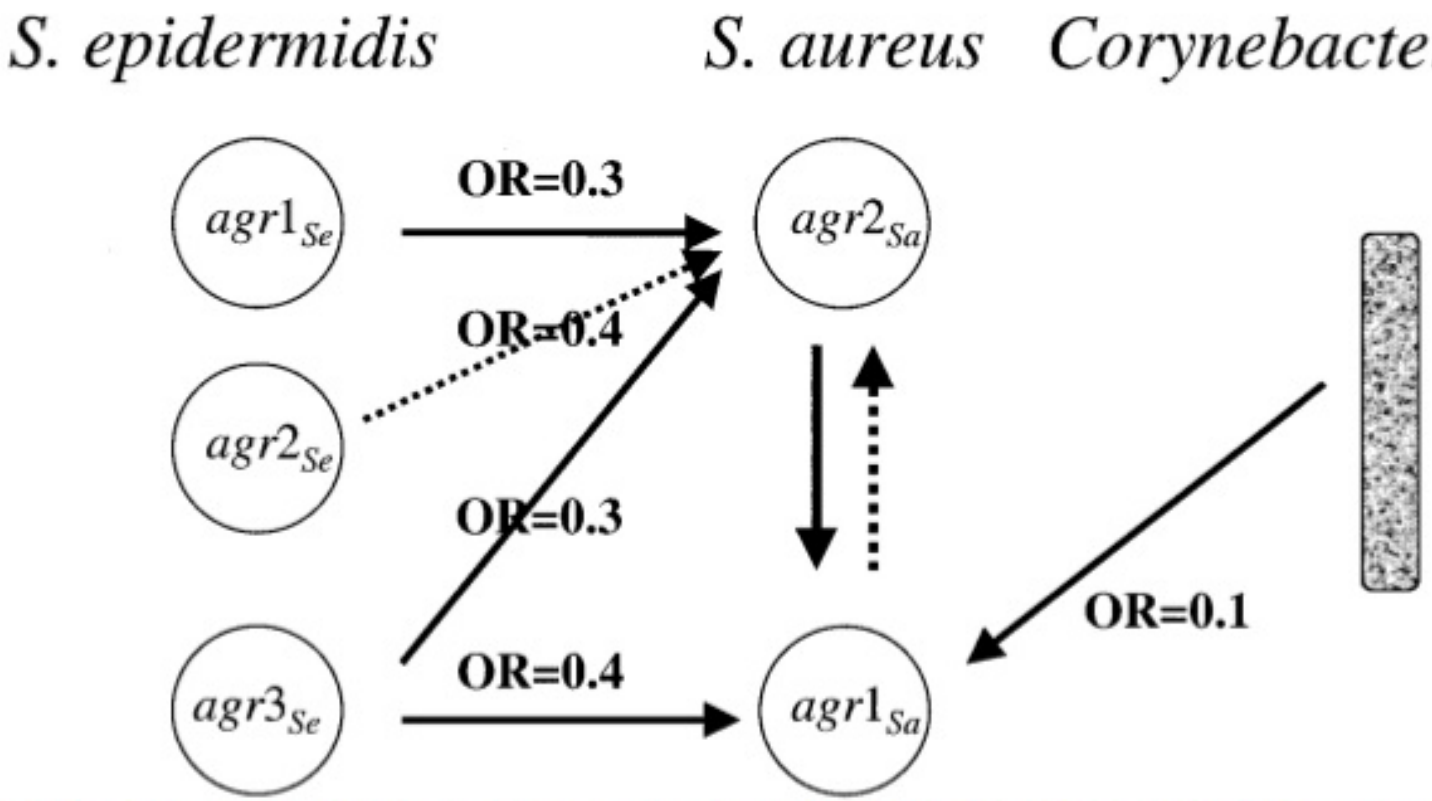
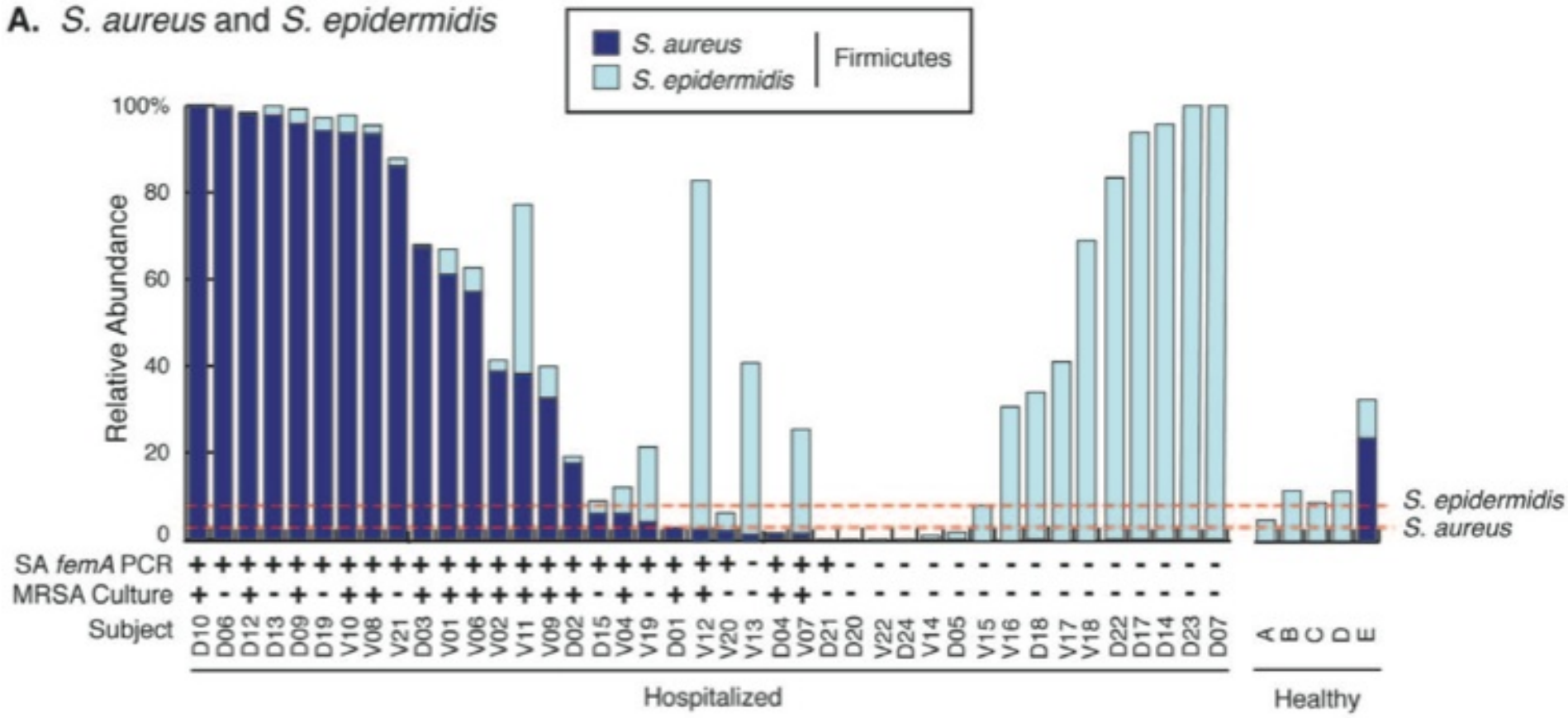


FIG. 2. Model of *agr*-dependent staphylococcal interference. Based on semiquantitative analysis of the aerobic nasal flora, a multiple logist gression model was used to analyze simultaneously the effect of *S. aureus* agr_{Sa} alleles, *Corynebacterium* spp., and *S. epidermidis* agr_{Se} alleles c *aureus* colonization. OR, odds ratio; threshold of 10^2 CFU for *S. epidermidis* and 10^4 CFU for *Corynebacterium* spp.

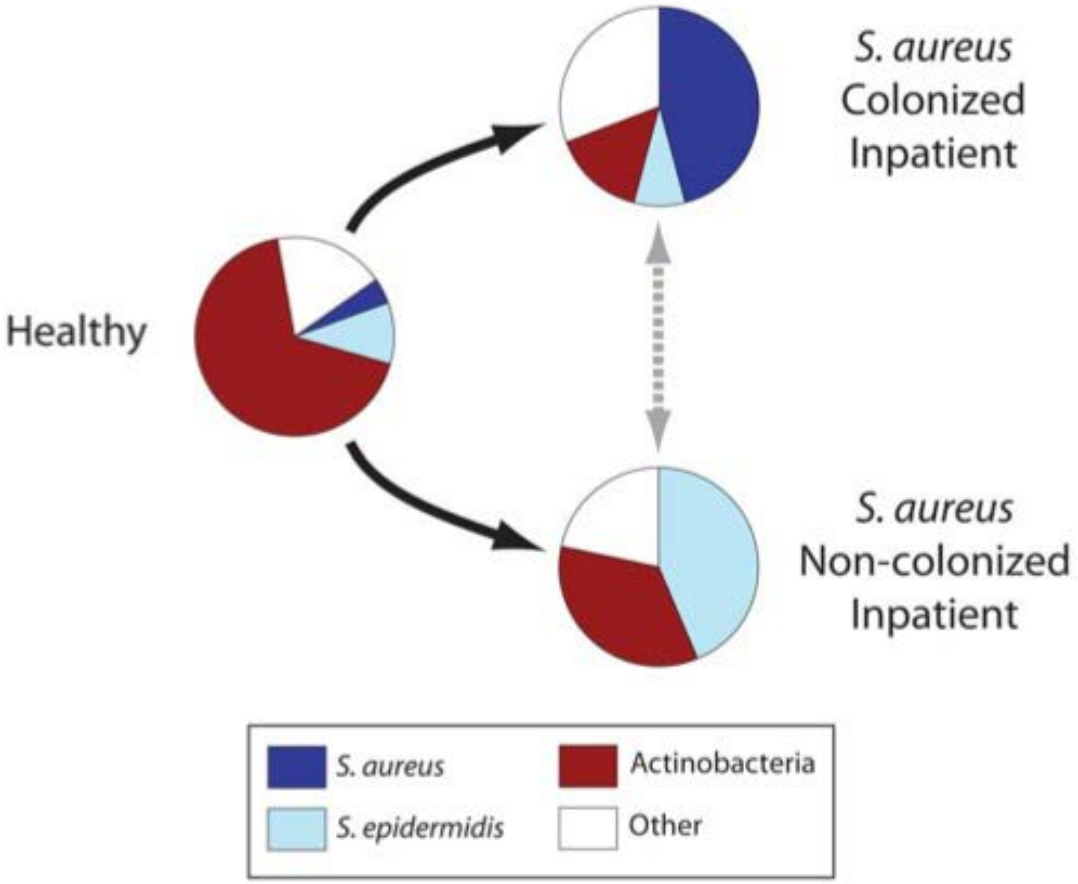
Lina et al. Bacterial Competition for Human Nasal Cavity Colonization: Role of Staphylococcal agr Alleles. Applied and Environmental Microbiology (2003)

Bacterial interference: *S.epidermidis vs S.aureus*



Frank DN et al. The Human Nasal Microbiota and Staphylococcus aureus Carriage. Plos One 2010

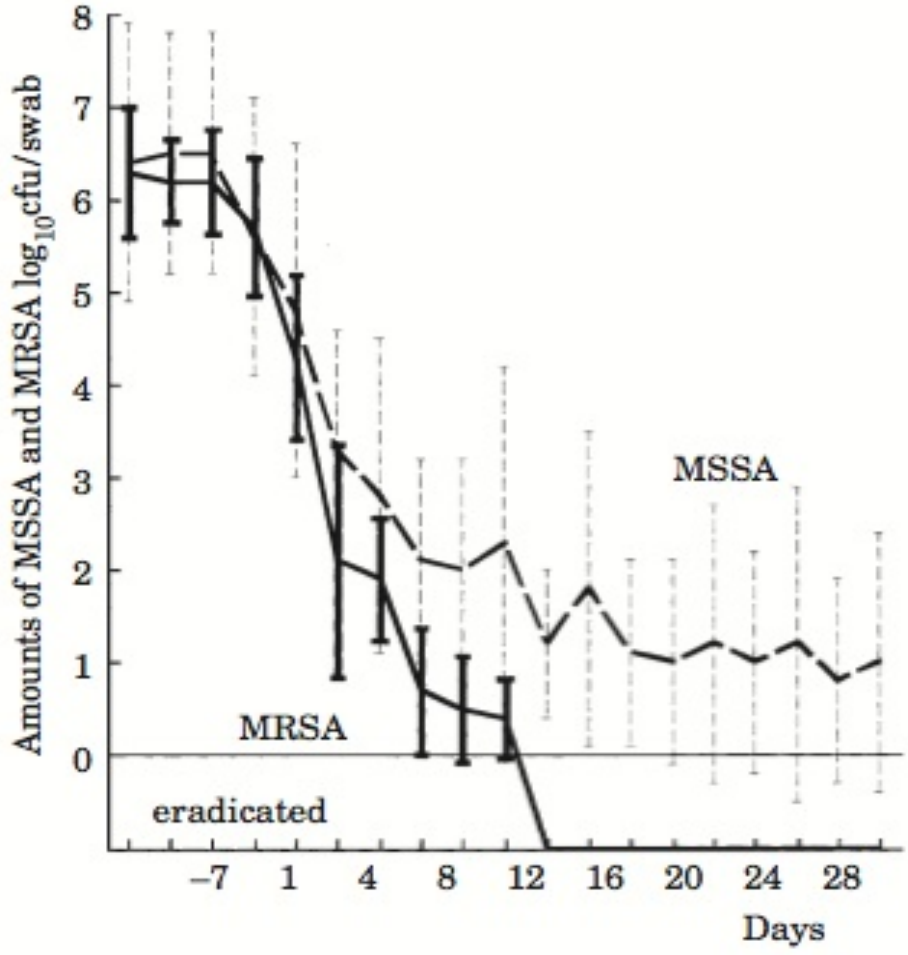
Bacterial interference



Frank DN et al. The Human Nasal Microbiota and Staphylococcus aureus Carriage. Plos One 2010

Bacterial interference

- **Implantation of a corynebacterium sp in the nose of 17 *S. aureus* carriers**
- **71% eradication of carriers by up to 15 inoculations (Fup: 3-35 months)**
- **similar doses of 0.9% NaCl or *S. epidermidis* : no effect**



Uehara et al. J. Hosp Infect 2000

Bacterial interference

- **Esp-secreting S. epidermidis in the nose of human volunteers correlates with the absence of S. aureus**
- **Purified Esp inhibits biofilm formation and destroys pre-existing *S. aureus* biofilms**

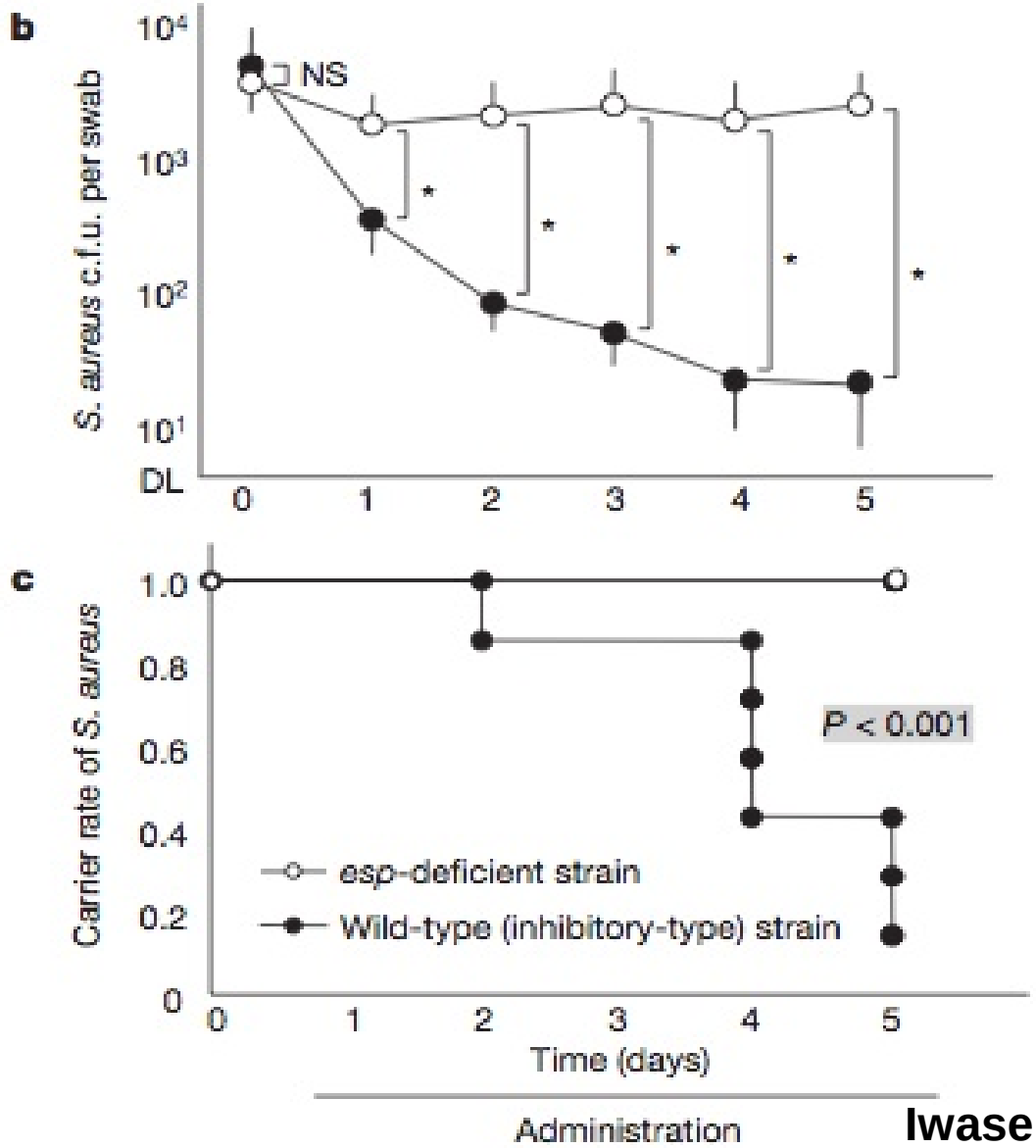
Table 1 | Odds ratios for *S. aureus* colonization in 88 volunteers (univariate analysis)

	S. aureus colonization			
Measurement	Yes ($n = 28$)	No ($n = 60$)	Odds ratio (95% CI)	*P*-value
Age (years)	22.2* (1.2)†	21.7* (1.3)†	1.10 (0.78–1.55)	0.58
Sex			2.61 (0.93–7.40)	0.07
Male	22	35		
Female	6	25		
Active smoking			0.33 (0.04–2.91)	0.32
Yes	1	6		
No	27	54		
Passive smoking			0.69 (0.26–1.83)	0.46
Yes	8	22		
No	20	38		
Allergy in nose			1.21 (0.49–3.00)	0.69
Yes	12	23		
No	16	37		
Allergy in eye			1.08 (0.30–3.95)	0.90
Yes	4	8		
No	24	52		
Allergy in skin			1.49 (0.51–4.36)	0.47
Yes	7	11		
No	21	49		
Antibiotic treatment in last month			0.69 (0.13–3.67)	0.67
Yes	2	6		
No	26	54		
Colonization of *S. epidermidis*			0.45 (0.06–3.36)	0.43
Yes	26	58		
No	2	2		
Colonization of inhibitory *S. epidermidis*			0.29 (0.11–0.76) 0.30 (0.11–0.80)‡	0.01
Yes	8	35		
No	20	25		

* Mean, †standard deviation, ‡multivariate analysis (adjusted for age, sex or smoking habits).

Iwase et al. Nature (2010)

Elimination effect of inhibitory *S. epidermidis* cells on *S. aureus* nasal colonization



Iwase et al. Nature (2010)

Outlines

- The different categories of carriers
- The various sites of carriage
- Factor affecting carriage
  - Environmental factors
  - Host / genetic factors
  - Bacterial factors
- Interventions : decontamination, vaccination, bacterial interference

Summary

- **The anterior nare is the main reservoir but the anatomical sites of carriage can vary according to the host and the bug**
- **The frequency and density of carriage depends on host (inherited) and environmental factors**
- **Carriage and disease isolates are genetically superimposable**
- **Numerous bacterial factor play a role in colonisation but none are specific to carriage isolates**
- **Basic rules of hygiene reduce nasal carriage**
- **Bacterial interference is a promising tool to combat carriage**

ACKNOWLEDGMENTS

Do not share personal items such as towels or razors.

Wash your hands frequently.

Shower immediately
after each practice and game.

Use clean towels each time you shower.

Launder clothes and towels after each use.