The adventures of Superbug; tracking the global spread of MRSA

Matthew Holden

wellcome trust sanger institute

Staphylococcus aureus

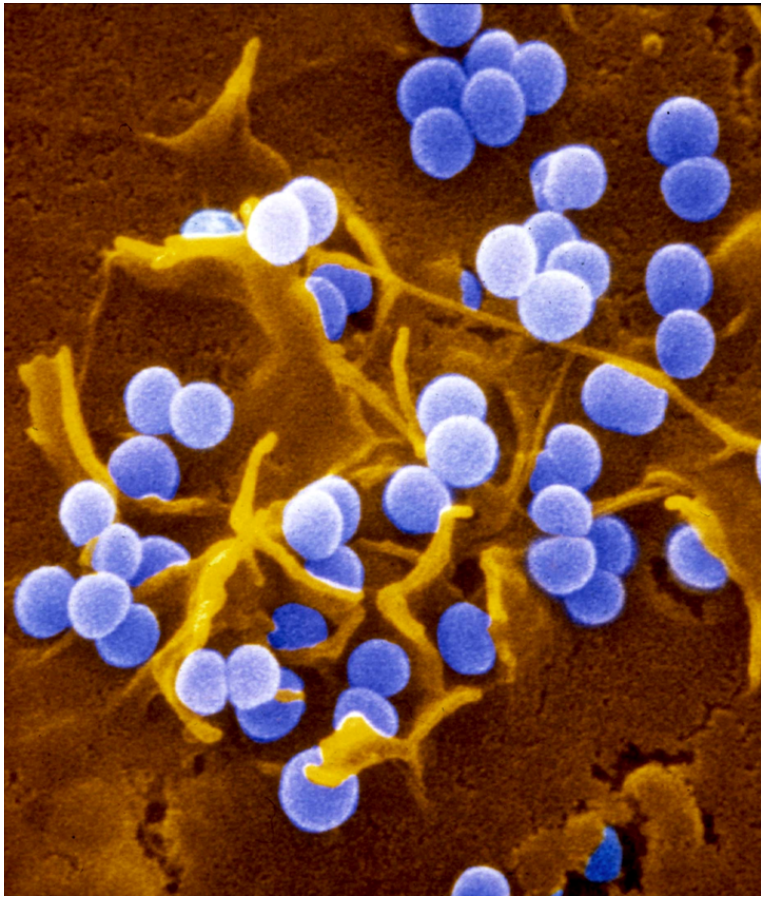
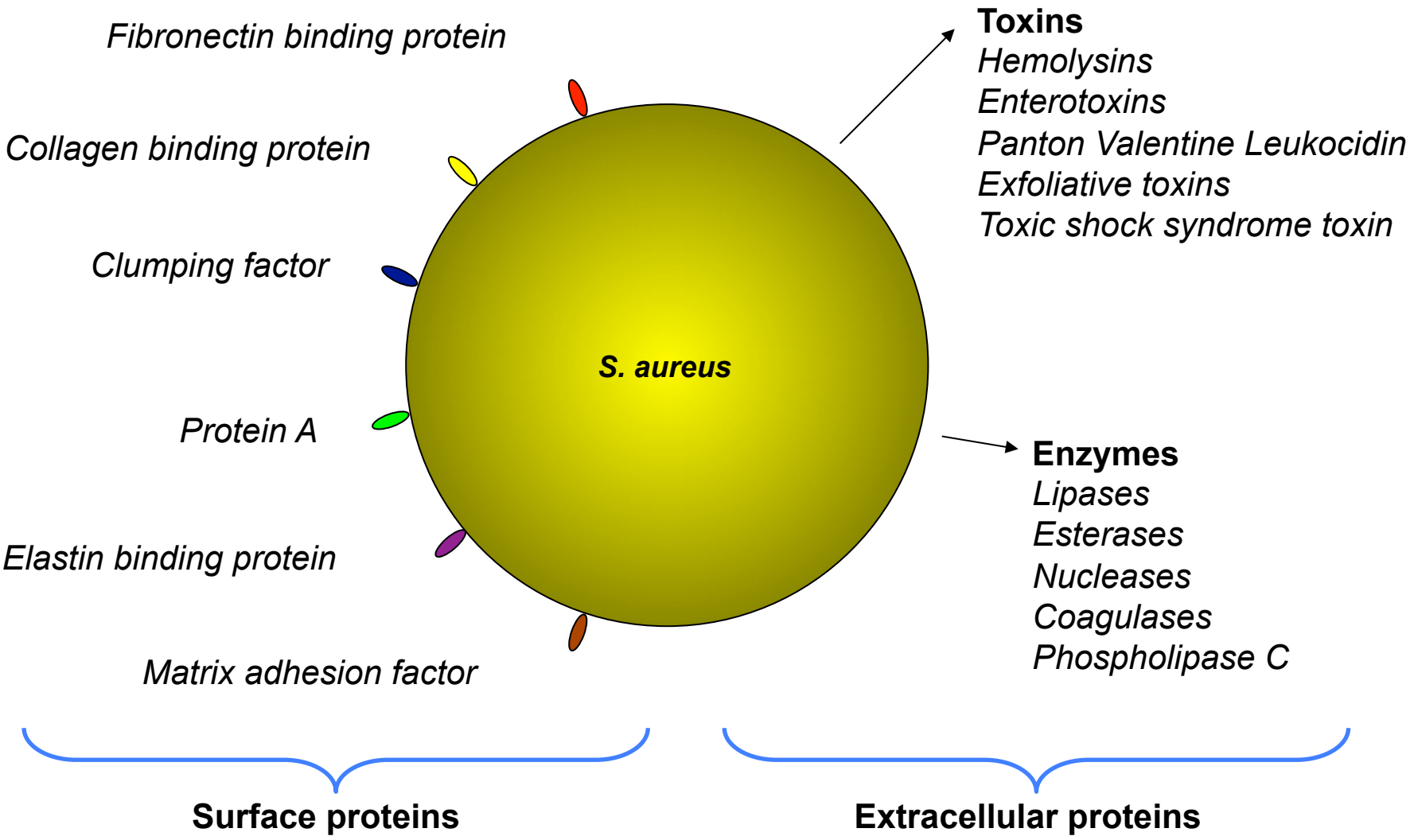


Image kindly provided by Sharon Peacock, Cambridge University

- Widespread Gram +ve bacteria
  - Natural flora of the skin
  - ~40% carriage in humans
  - Farm animals and pets
- Versatile pathogen associated with a wide range of diseases
  - Minor wound infections
  - Food poisoning
  - Toxic shock syndrome
  - Endocarditis
  - Haemolytic pneumonia
- Complex pathology

wellcome trust sanger institute

Virulence factors



wellcome trust sanger institute

Staphylococcus aureus

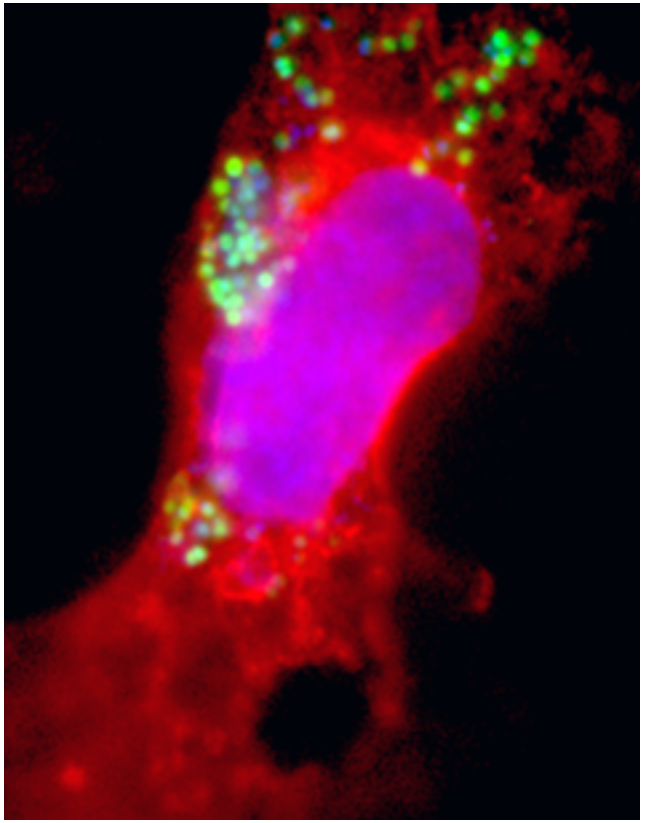
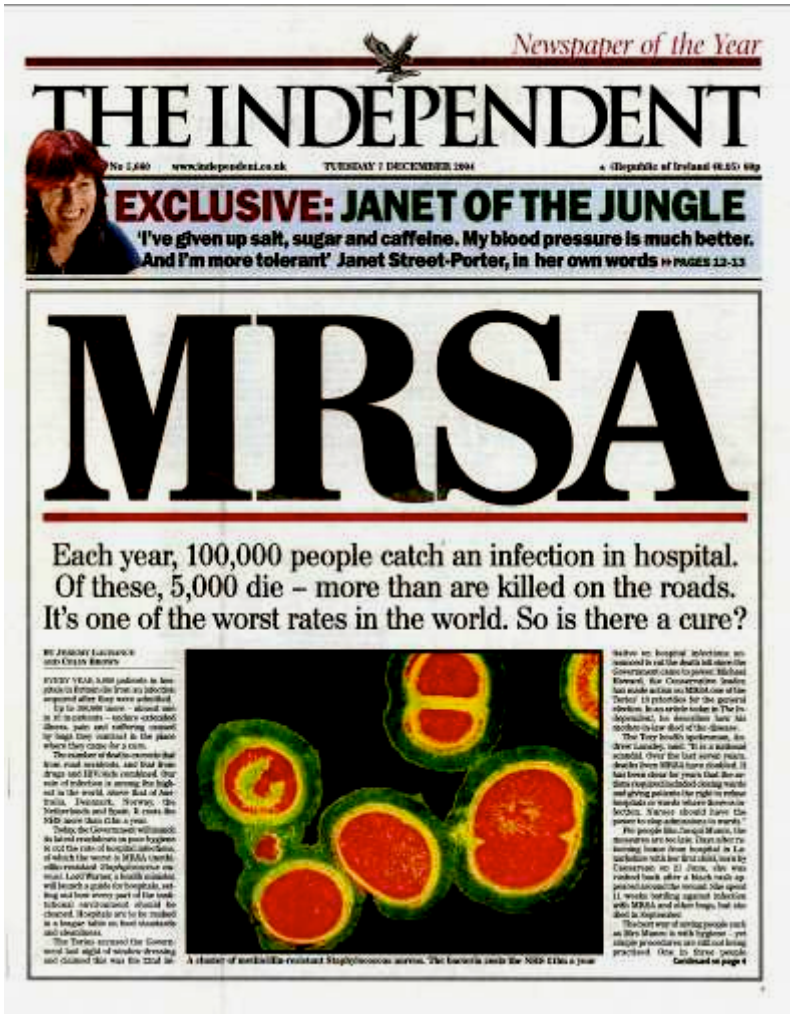


Image kindly provided by Phil Hill, Nottingham University

- Hospital-acquired infections
  - In the USA 1 million cases of hospital acquired *S. aureus* infections a year
  - Prolonged hospital stay
    - 2.5 times longer
  - Increased costs
    - £1 billion a year
- Community-acquired infections
  - Invasive
  - Low levels of drug resistance
  - Increase in the levels of infection

wellcome trust sanger institute

Antibiotic resistance


Newspaper of the Year

THE INDEPENDENT

EXCLUSIVE: JANET OF THE JUNGLE

'I've given up salt, sugar and caffeine. My blood pressure is much better. And I'm more tolerant' Janet Street-Porter, in her own words

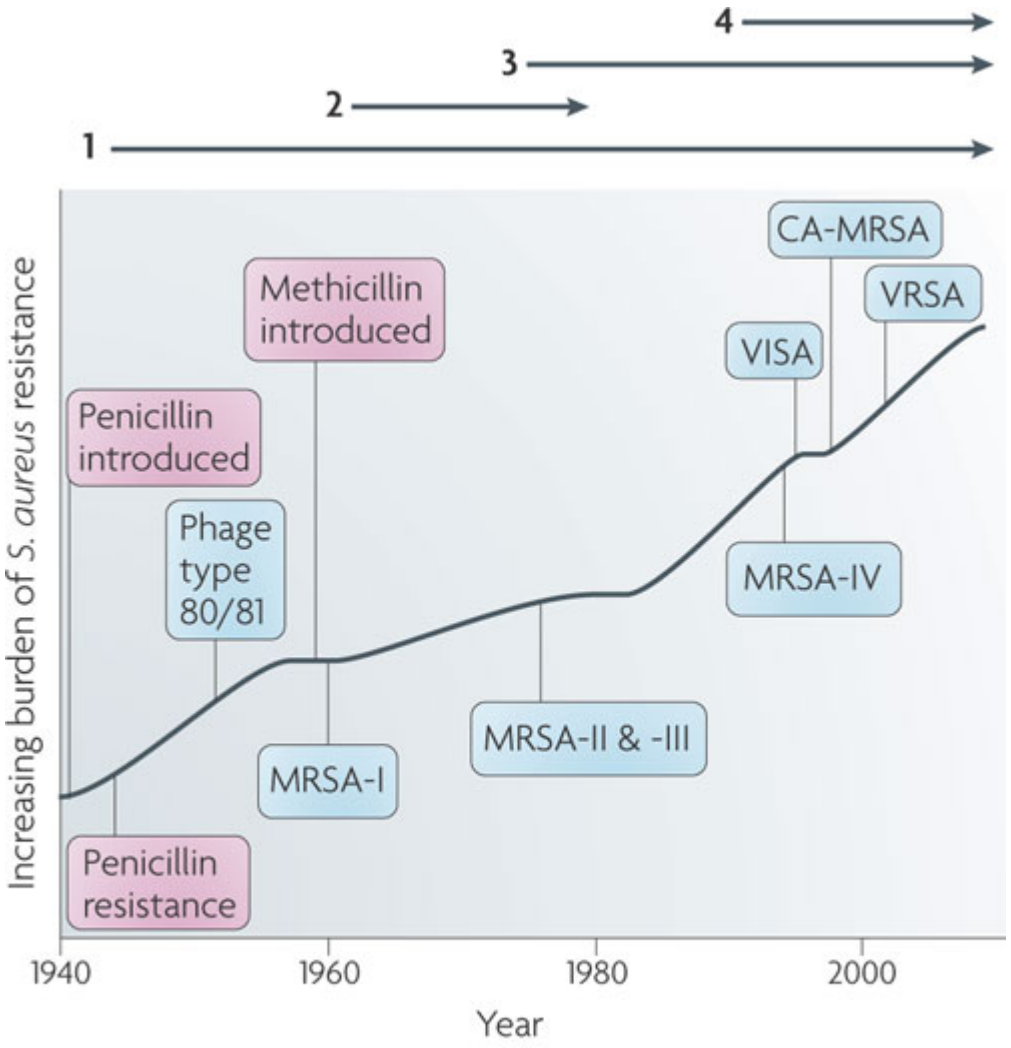
MRSA

Each year, 100,000 people catch an infection in hospital. Of these, 5,000 die – more than are killed on the roads. It's one of the worst rates in the world. So is there a cure?

- Antibiotic resistance
  - **MRSA** Methicillin resistant *S. aureus*
  - **VISA** Vancomycin insensitive *S. aureus* (MIC, 8-16 μg ml^{-1})
  - **VRSA** Vancomycin resistant *S. aureus*
- On the increase….

wellcome trust sanger institute

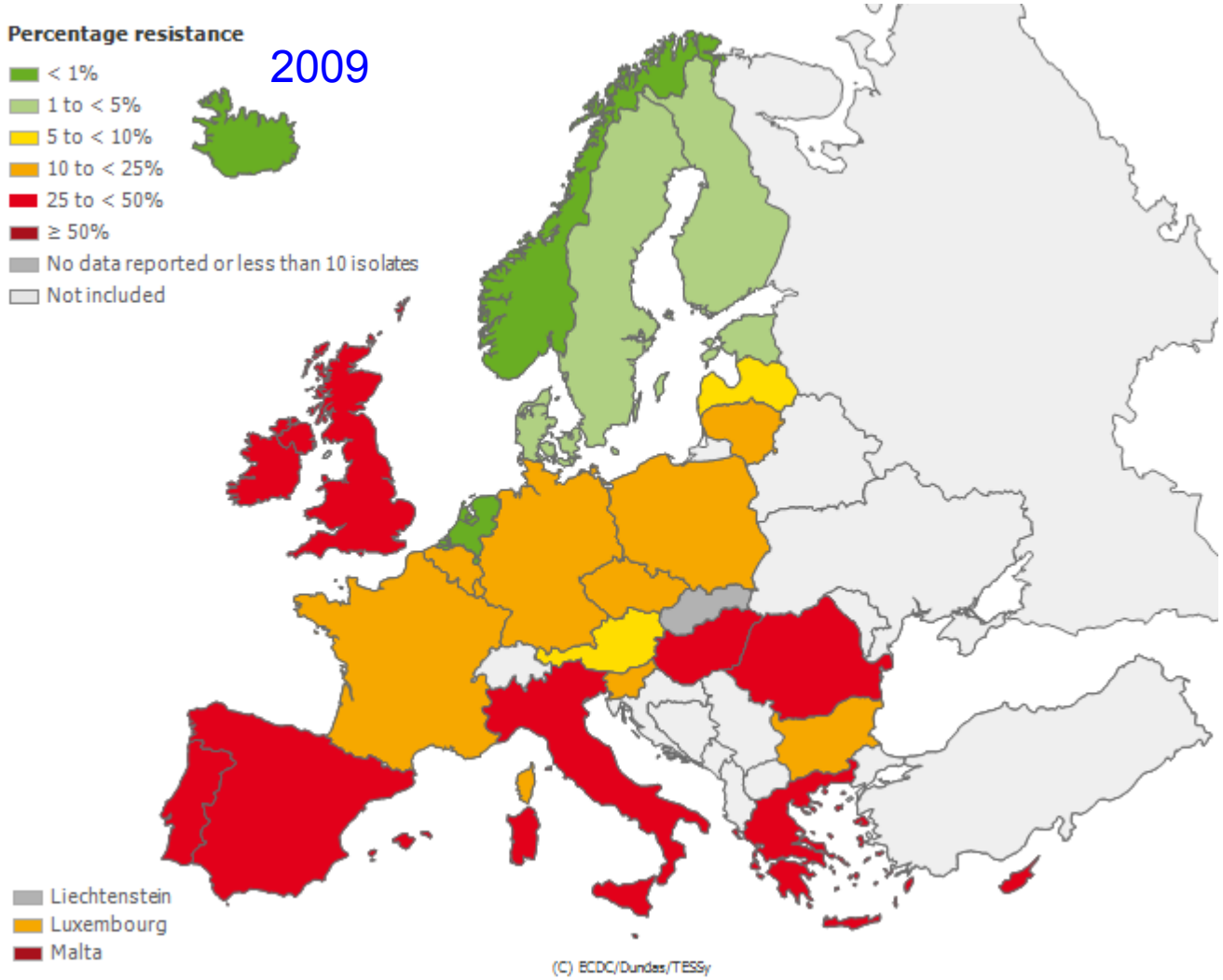
Waves of drug resistant *S. aureus*



Chambers and De Leo Nature Reviews | Microbiology

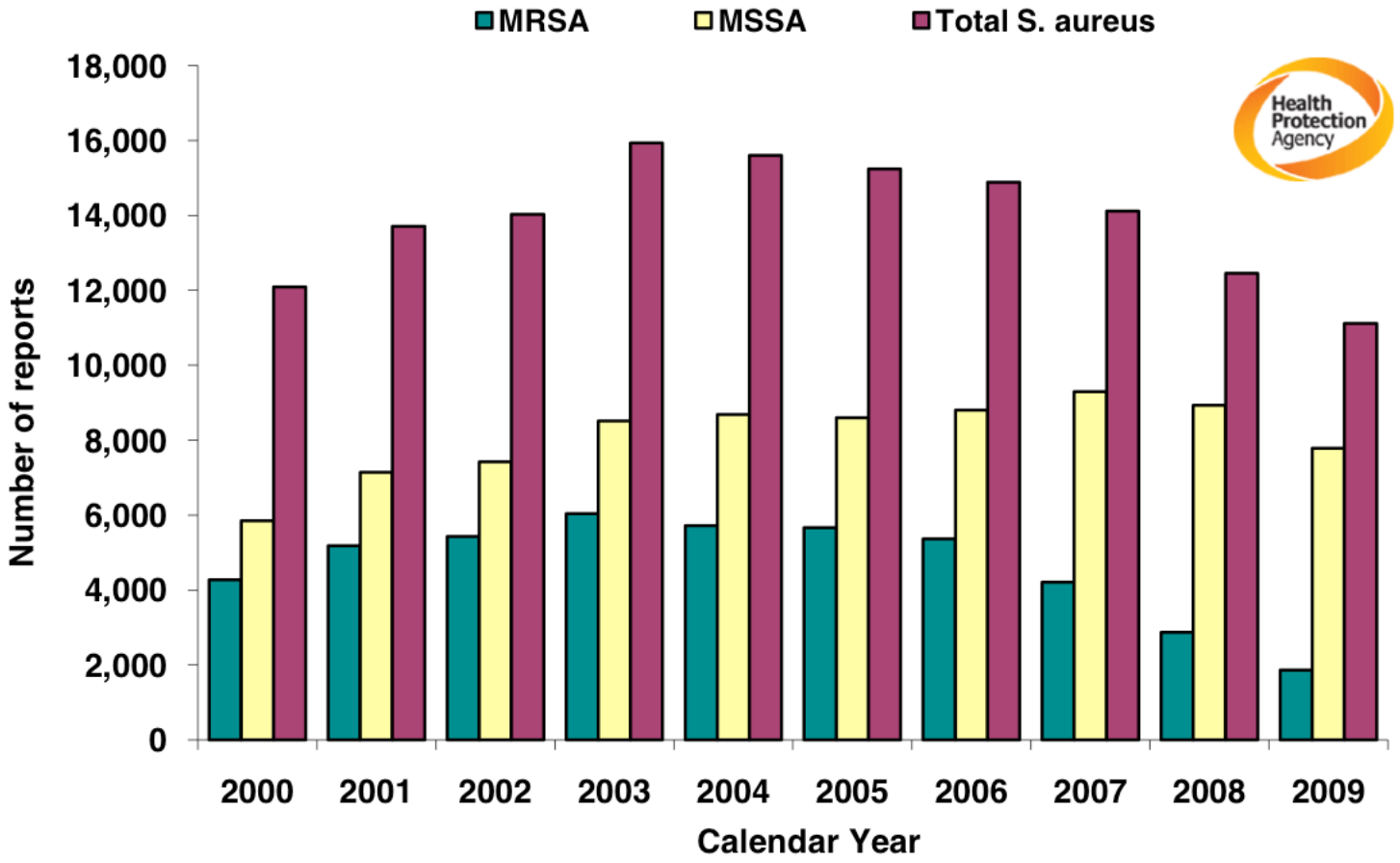
wellcome trust sanger institute

Spread of MRSA in Europe 2001-2009



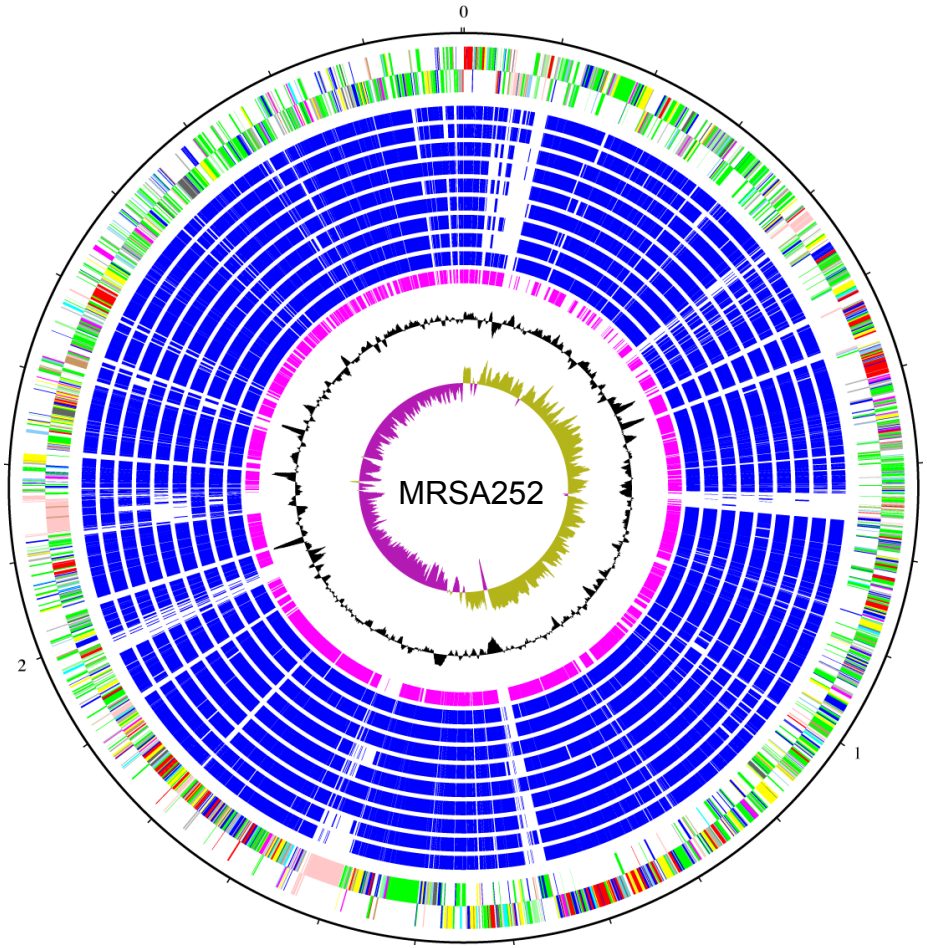
Voluntary reporting of *Staphylococcus aureus* bacteraemia in England, Wales and Northern Ireland, 2009

Figure 1: Trend in *Staphylococcus aureus* bacteraemia laboratory reports and meticillin susceptibility (voluntary reporting scheme): England, Wales and Northern Ireland 2000-2009



wellcome trust sanger institute

What can genomics do for superbugs?



- Complete gene map
  - Unravel the mechanisms of disease
    - Look for the genes of proteins that attack the host - virulence factors
  - Identify new targets for antimicrobial therapies
    - New drugs
    - Vaccine
- How do superbugs evolve?
  - Antibiotic resistance
    - Mobile cassettes
  - Virulence factors
    - Bacteriophage

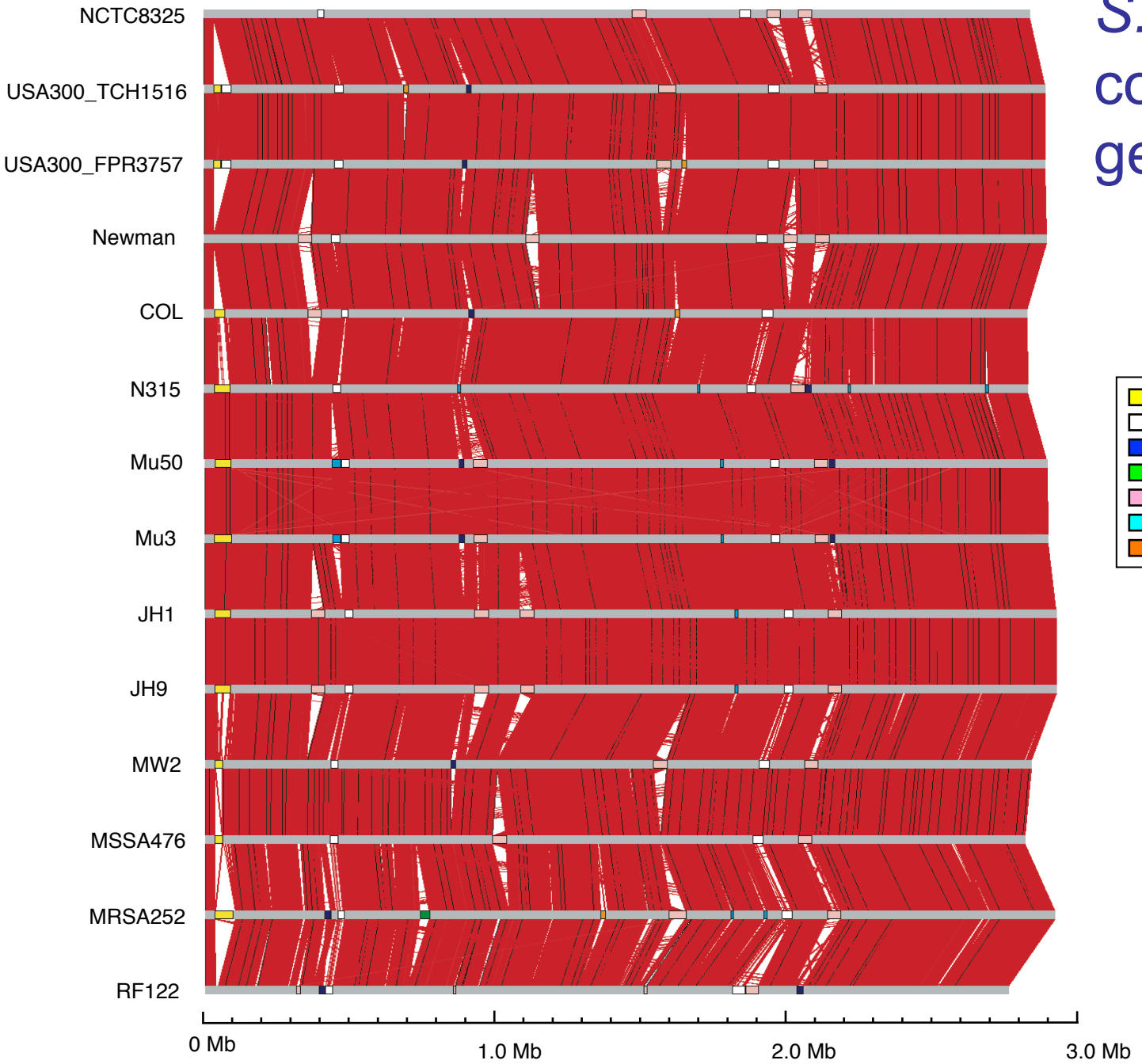
wellcome trust sanger institute

Sequenced *Staphylococcus aureus* strains

N315	Hospital-acquired MRSA	Kuroda *et al*. (2001)
Mu50	Hospital-acquired VISA	Kuroda *et al*. (2001)
MW2	Community-acquired MRSA	Baba *et al*. (2002)
MRSA252	Hospital-acquired MRSA	Holden *et al*. (2004)
MSSA476	Community-acquired MSSA	Holden *et al*. (2004)
COL	Early MRSA from the 1960s	Gill *et al*. (2005)
USA300_FPR3757	Community-acquired MRSA	Diep *et al.* (2006)
NCTC8325	Lab strain	Gillapsy *et al.* (2006)
JH1	Hospital-acquired MRSA	Mwangi *et al.* (2007)
JH9	VISA derivative of JH9	Mwangi *et al.* (2007)
RF122	Bovine isolate	Herron-Olson (2007)
USA300_TCH1516	Community-acquired MRSA	Highlander *et al.* (2007)
Newman	Hospital-acquired MSSA	Baba et al. (2008)
Mu3	Hospital-acquired VISA	Neoh *et al*. (2008)

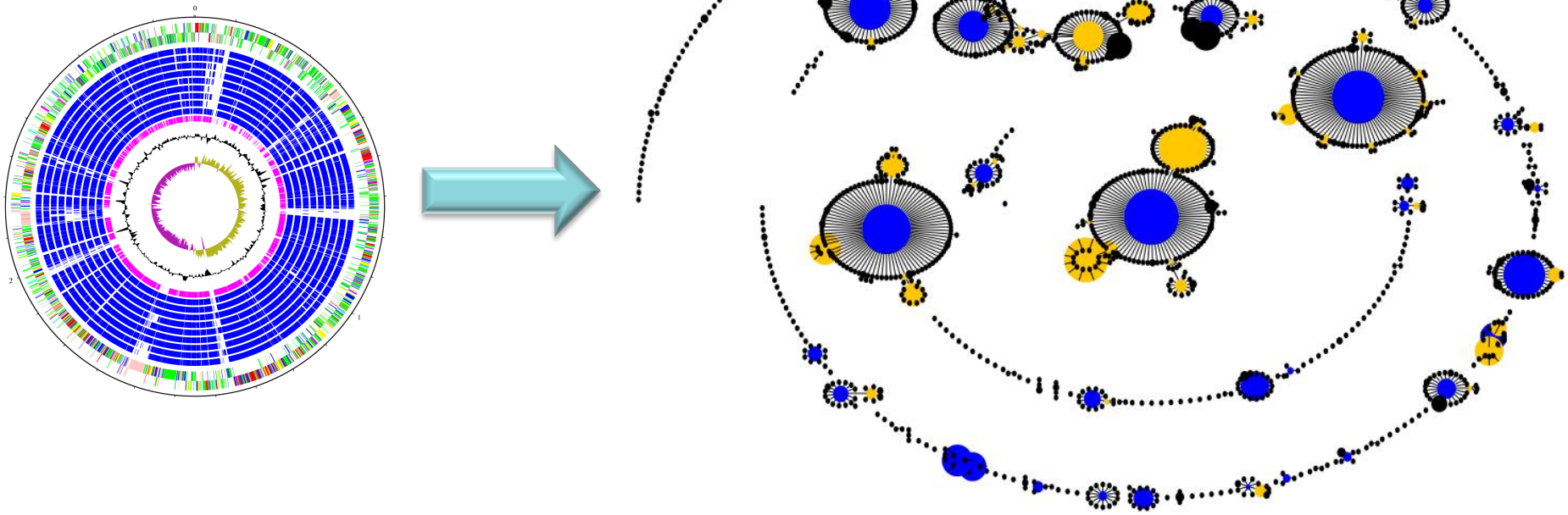
wellcome trust sanger institute

S. aureus comparative genomics



wellcome trust sanger institute

Genomics: from individual to the population



wellcome trust sanger institute

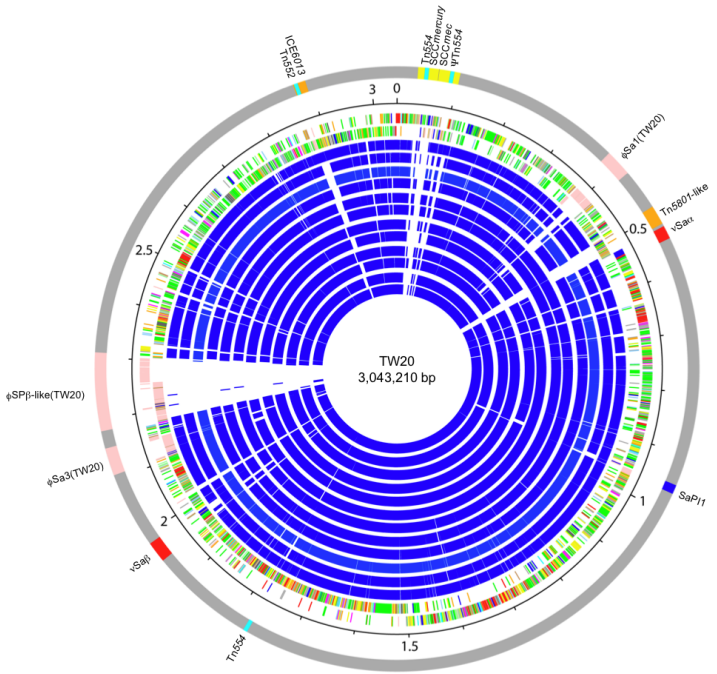
The *S. aureus* ST239 lineage

Collaboration with Hermínia de Lencastre, Ed Feil, Sharon Peacock

- The most common worldwide strain of MRSA
  - ~90% of Asian strains in the MLST database
  - Significant in 26 countries outside Asia
    - Particularly Brazil
    - Recent outbreak in Guy's and St Thomas', London (TW20)

- The assembled collection includes 62 isolates:
  - Wide geographical range:
    - Europe, North and South America, Asia, Australia
  - 20 year temporal range
    - 20 isolates from a 7 month hospital transmission study

- Collection sequenced using Illumina multiplexing
  - High resolution genotyping and pan-genome diversity



Holden et al. (in press) Genome sequence of a recently emerged highly-transmissible, multi-antibiotic and antiseptic resistant, variant of methicillin-resistant *Staphylococcus aureus* (MRSA) sequence-type 239 (TW) *J Bacteriol*

  - Harris *et al*. (2010) Evolution of MRSA during hospital transmission and intercontinental spread. *Science*

wellcome trust sanger institute

Whole genome sequencing as a typing tool

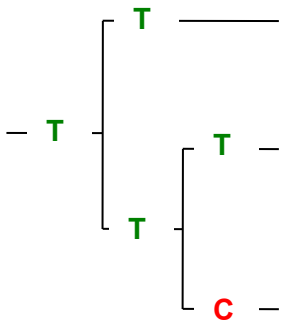
Illumina reads mapped to a reference genome

Single Nucleotide Polymorphisms identified

Gather SNPs from other isolates

Phylogenetic tree

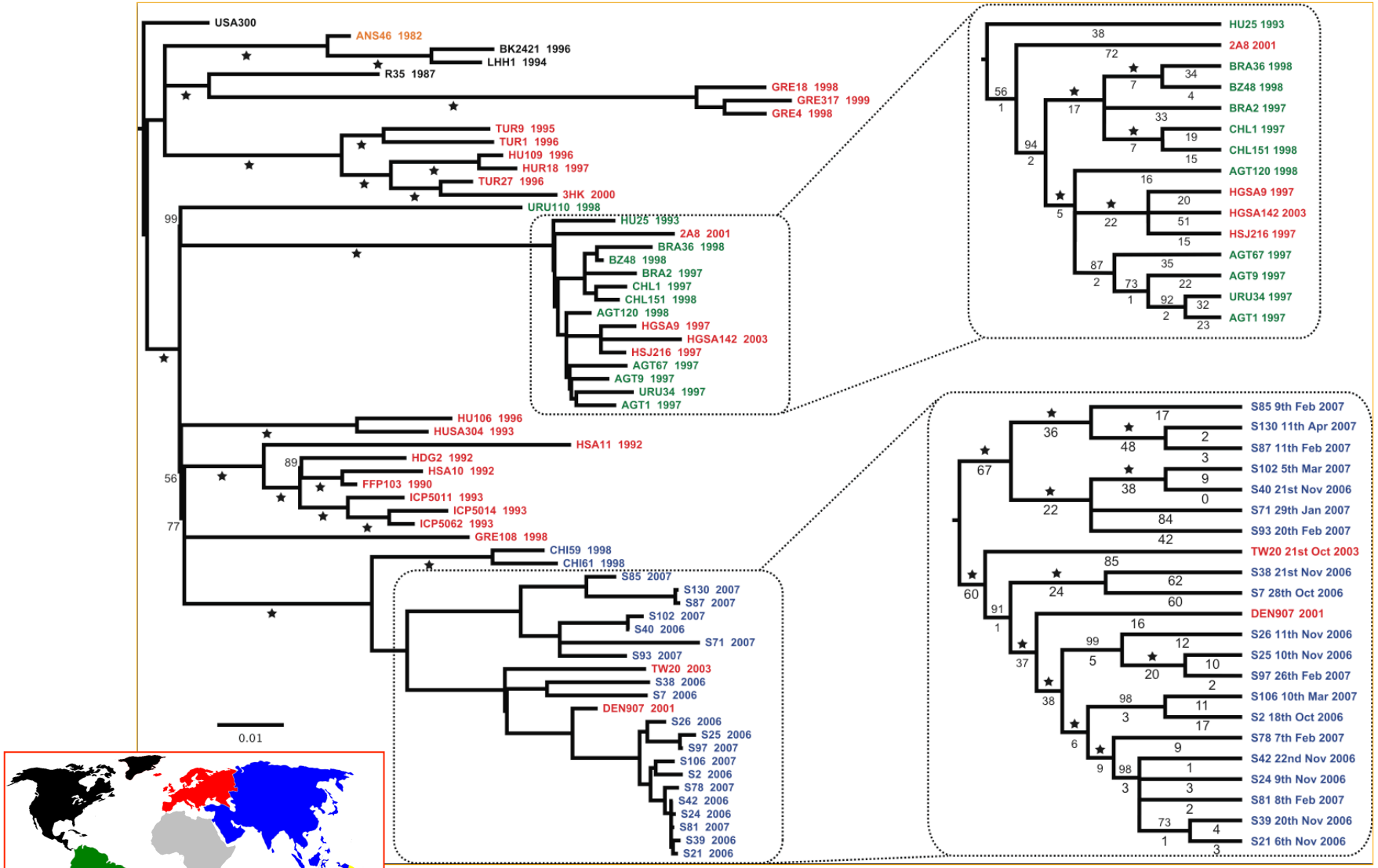
All SNPs used to construct a tree



A single SNP can distinguish closely related isolates

wellcome trust sanger institute

Geographic structure within ST239



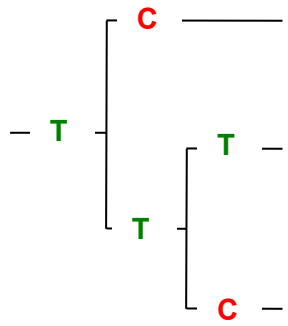
wellcome trust sanger institute

Genetic variation within the lineage

wellcome trust sanger institute

Clinical practice is shaping the genetic makeup of ST239

Phylogenetic tree



Detection of homoplasies

SNPs that occur independently

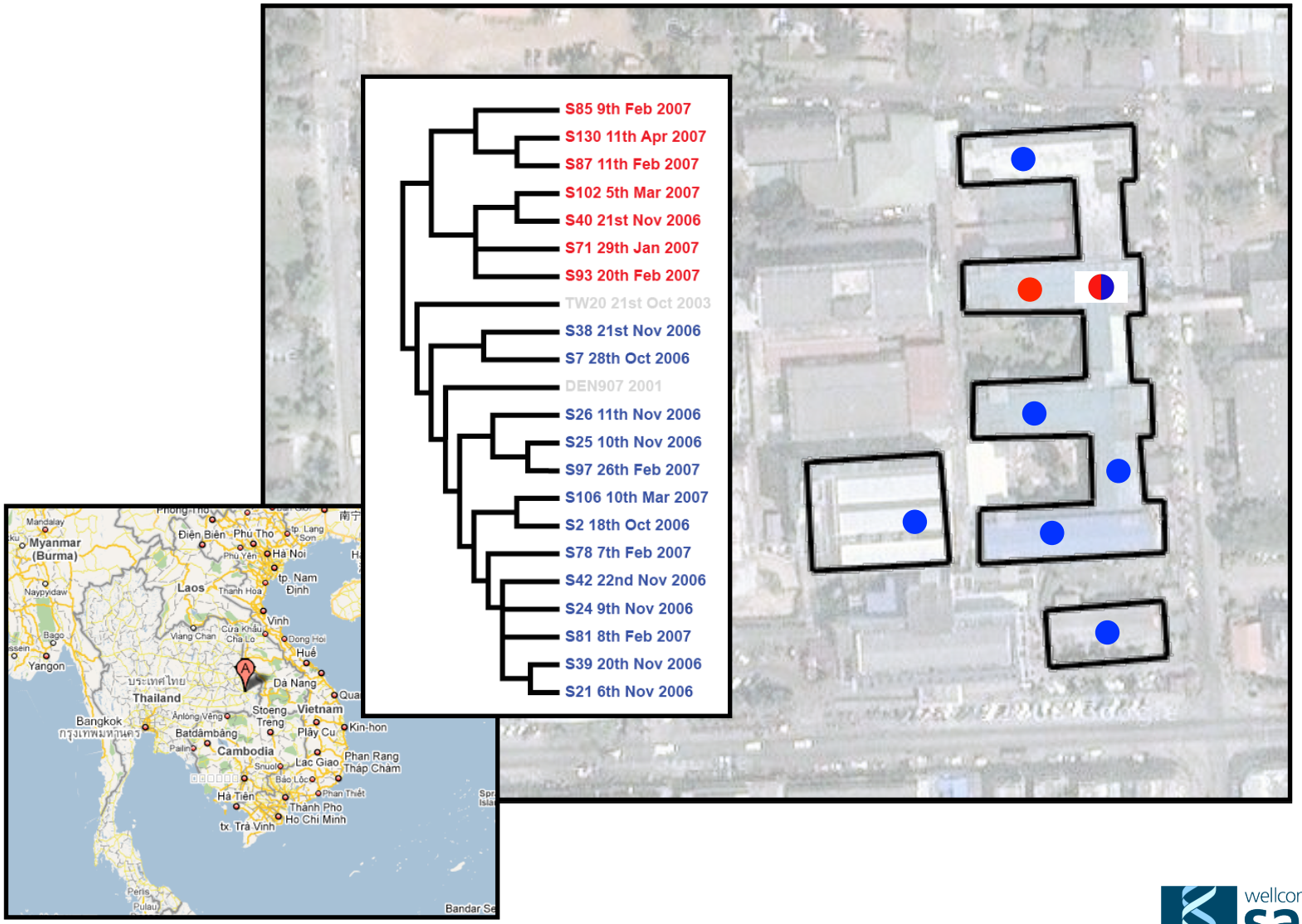
The same SNP found in different parts of the tree

Selective pressure has lead to the same SNP as the substitution may be advantageous

SNP position	Locus affected	No. of "branches"	SNP	Substitution	Antibiotic
7254	DNA gyrase subunit A GyrA	2	T => G	Ser84Ala	
7255	DNA gyrase subunit A GyrA	8	C => T	Ser84Leu*	quinolone
7266	DNA gyrase subunit A GyrA	4	G => A	Lys88Glu	quinolone
133864	immunoglobulin G binding protein A precursor	2	G => A	Synonymous	-
134787	92 bp upstream of immunoglobulin G binding protein A	2	G => T	Intergenic	-
278498	129 bp upstream of putative acetyl-CoA transferase	2	T => C	Intergenic	-
436474	34 bp upstream of putative dioxygenase	2	C => T	Intergenic	-
594883	tetrapyrrole (corrin/porphyrin) methylase family protein	2	C => T	Pro49Ser	-
657696	DNA-directed RNA polymerase beta chain protein RpoB	4	C => A	Asp471Glu	rifampin
657724	DNA-directed RNA polymerase beta chain protein RpoB	5	C => A	His481Asn	rifampin
657869	DNA-directed RNA polymerase beta chain protein RpoB	2	C => T	Ser529Leu	rifampin
666536	translation elongation factor G	2	T => A	Leu461Lys	fusidic acid
666537	translation elongation factor G	2	T => A	Leu461Lys*	fusidic acid
681826	48 bp upstream of serine-aspartate repeat-protein C	2	C => A	Intergenic	-
862898	putative membrane protein	2	A => C	Ser160Ala	-
1130135	63 bp upstream of FolD bifunctional protein	2	G => T	Intergenic	-
1138698	phosphoribosylglycinamide formyltransferase PurN	3	T => A	Leu174Met	-
1172434	50 bp upstream of probable manganese transport protein	3	T => G	Intergenic	-
1172436	52 bp upstream of probable manganese transport protein	2	T => C	Intergenic	-
1172444	60 bp upstream of probable manganese transport protein	2	C => G	Intergenic	-
1206826	ribonuclease HIII	2	C => T	Glu199Lys	-
1261219	isoleucyl-tRNA synthetase	2	G => T	Val588Phe	mupirocin
1448063	topoisomerase IV subunit A GrlA	4	T => C	Ser80Phe	quinolone
1524413	dihydrofolate reductase type I Dfr B	2	T => C	His150Arg	trimethoprim
1524566	dihydrofolate reductase type I Dfr B	4	A => T	Phe99Tyr	trimethoprim
1524789	dihydrofolate reductase type I Dfr B	2	G => A	Synonymous	-
1525796	thymidylate synthase	3	G => A	Synonymous	-
1525817	thymidylate synthase	3	G => A	Synonymous	-
1525832	thymidylate synthase	3	G => A	Synonymous	-
1640281	glyoxalase/bleomycin resistance protein	2	T => G	Synonymous	-
1689862	putative transcriptional repressor CcpN	2	C => T	Synonymous	-
1755814	probable cell wall amidase Lyt H	2	A => G	Pro63Ser	-
1921379	bifunctional riboflavin biosynthesis protein RibD	2	G => T	Asn208Lys	-
2334865	protein SprT-like	2	G => A	Ser43Phe	-
2753531	458 bp upstream of conserved hypothetical protein	2	A => T	Intergenic	-
2828688	200 bp downstream of putative exported protein	3	T => C	Intergenic	-
2828714	226 bp downstream of putative exported protein	3	G => T	Intergenic	-
2859765	39 bp upstream of O-acetyltransferase OatA	2	C => T	Intergenic	-

wellcome trust sanger institute

Sappasithiprasong Hospital



wellcome trust sanger institute

Summary

- *S. aureus* generate diversity by a variety of means
  - Core and accessory genomes
  - Horizontal gene transfer is important
  - Evidence of the rapid movement of virulence and drug resistance determinants
  - Point mutations - SNPs

- Whole genome sequencing provides a high-resolution view of the epidemiology and microevolution
  - Geographical structuring and temporal spread
  - Evidence of intercontinental movement of the lineage
  - Potential to trace person-to-person transmission

- Reducing costs and improving performance of sequencing
  - Future technology transit, from lab to bedside?

wellcome trust sanger institute

S. aureus ST239 Collaborations

Stephen Bentley

Simon Harris

Julian Parkhill

Michael Quail

University of Bath

Ed Feil

Mahidal-Oxford Tropical Medicine Unit, Bangkok

Sharon Peacock

Emma Nickerson

Narisara Chantratita

Nick Day

University of London

Jodi Lindsay

King's College

Jonathan Edgeworth

Universidade Nova de Lisboa

Hermínia de Lencastre

Susana Garadete

Ana Tavares

Imperial College

Brian Spratt

David Aanensen