# Exploring the evolution of MRSA with Whole Genome Sequencing

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Introduction

MRSA:

❖ Remains a leading cause of hospital-acquired infection(Healthcare-associated , **HA-MRSA**)

❖ Also causing community outbreaks (Community-associated, **CA- MRSA**).
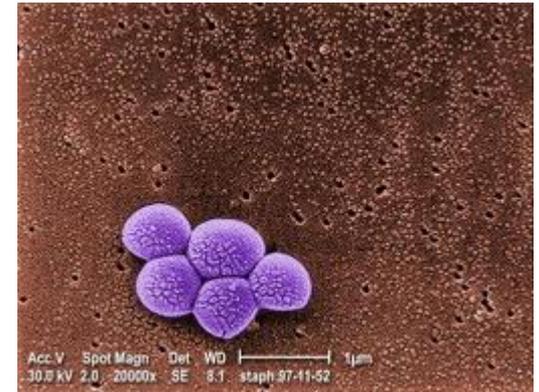

The Epidemic has been driven by a limited number of clones,

such as CA-MRSA   ST8(USA300)   in North America.
ST80 and ST45 in Europe
ST30 and ST59 in Asia

HA-MRSA   ST239 ST22 ST36 CC5 CC8   worldwide prevalence

(Huang TW, et al, J Bacteriol. 2012;Brady, et al, J. Clin. Microbiol. 2007)

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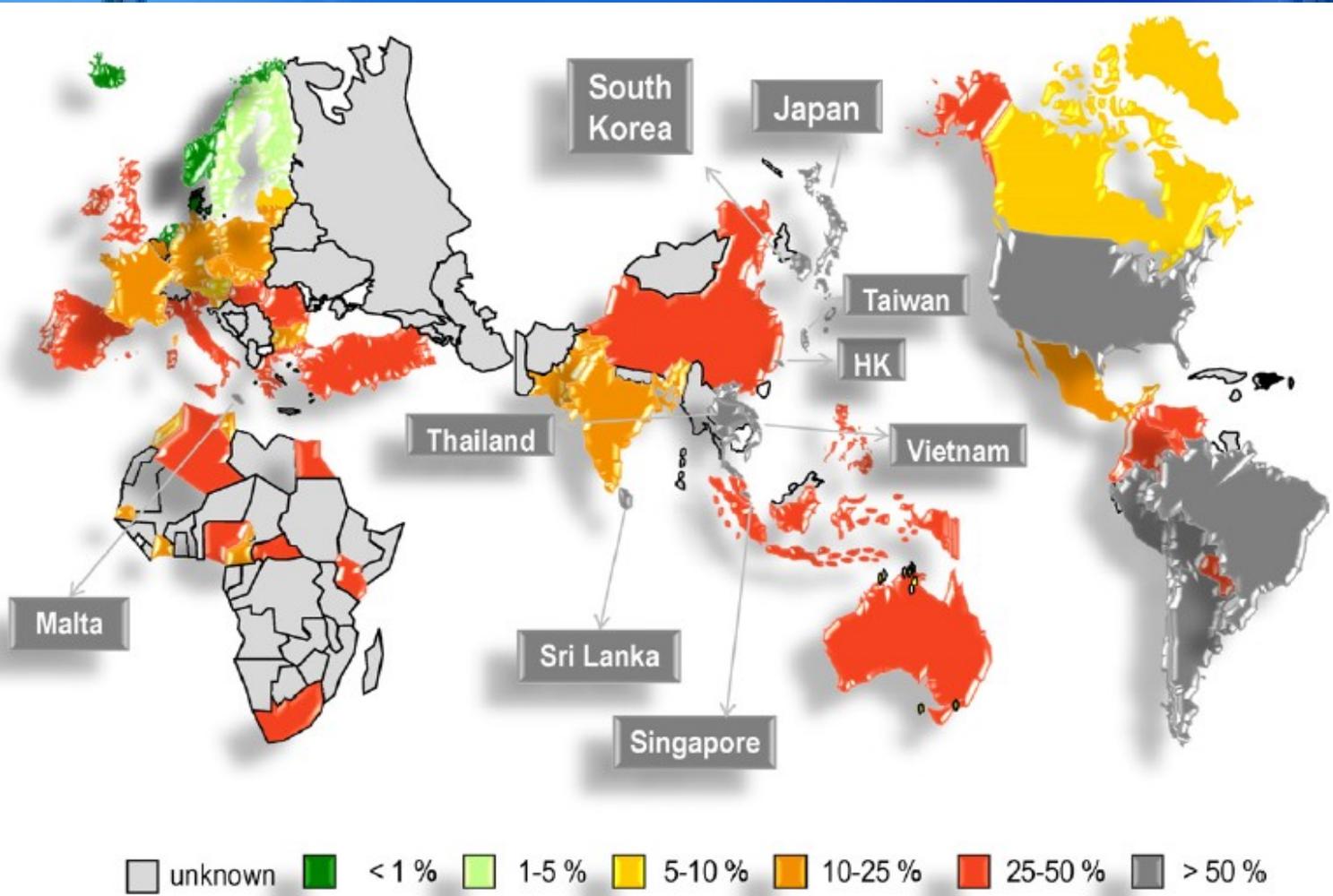
Worldwide prevalence of HA-MRSA
RATES: HA-MRSA as proportion of SA infections

Conventional typing methods used for phylogeny and evolution

MLST

❖ 7 housekeeping genes

❖ classifies MRSA strains into groups that reflect **phylogeny**, **population structure** and **evolutionary history**

Limitations of Conventional typing methods (MLST PFGE spa in combination)

❖ **Insufficiently discriminatory** within a special lineage

❖ Fail to reveal the fine details of

1. DNA polymerases copying mistakes

2. point mutations

3. recombination events

4. mobile elements gained and lost

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Comparison of the MRSA typing techniques

Technique	Set-up cost	Cost per isolate	Current availability	Time to results	Data analysis	Data transfer ability	Level of resolution
PFGE	Low	£4-7	Local and reference laboratories	2-3 days	Minimal	Limited	Lineage
MLST	High	£20	Research and reference laboratories	Days	Moderate	Yes-widely	Lineage
spa	High	£3-5	Local and reference laboratories	24 h	Moderate	Yes-widely	Lineage
WGS	**High**	**~£100**	**Research laboratories**	**Real time[a]**	**High**	**Being addressed**	**Base pair**

[a] Third generation sequencing platforms

WGS makes it possible to determine when sequences really are identical, or, if not, show how much they are different.

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❖ **Evolution and Phylogeny**

enhance understanding of the effects of selective pressures(e.g. antibiotic exposure) on bacterial populations

❖ **Outbreaks investigation**

genealogical analysis identify possible transmission with least supporting epidemiological information

❖ **Phenotypic predictions**

identification of mutations associated with unusual antibiotic susceptibility patterns, strain growth rates

Final Aim

Infection control and prevention

Comparison of sequencing technologies

	Sequencing technology platform		
	First generation	**Second generation**	**Third generation**
Resolution	Average of multiple DNA copies	Average of multiple DNA copies	Single molecule
Read length generated	800-1000 bp	<400 bp	1000-10,000 bp
Financial cost per base	High	Low	Moderate
Financial cost per run	Low	High	Low
Sample preparation	Moderate	Complex	Variable
Time to result	Hours	Days	Minutes to hours
Platform	**ABI 3730XL**	**Illimina GA ROCHE-454 SOLiD** MiSeq Ion Torrent	**PacBio RS**

MRSA genome
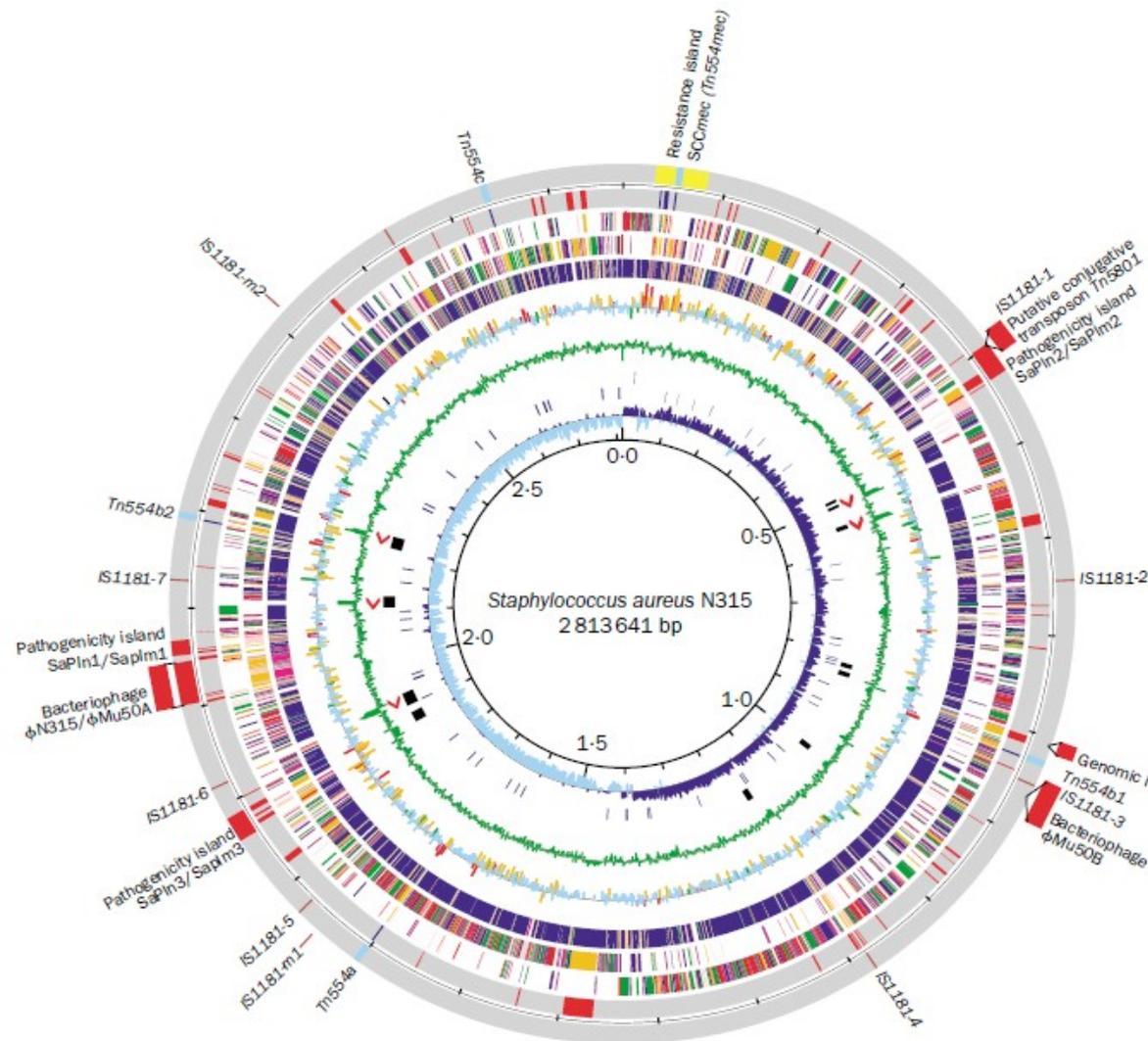
❖ **The circular genome of MRSA :**
2.8 million nucleotides

❖ 10% of the genome that vary between different lineages.
'core variable genome'

❖ 10% - 20% of the genome consists of
'mobile genetic elements'

(Kuroda M, et al, Lancet 2001)

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Evolution of MRSA During Hospital Transmission and Intercontinental Spread

(Harris SR, et al, Science 2010)

❖ 1. Population structures: demonstrated geographical clustering of isolates

❖ 2. Distinguish possible transmission from endemic infection

❖ 3. Estimates of mutation rate

❖ **Target Lineage : ST239**

   ❖ accounts for most of the HA-MRSA strains in mainland Asia ; circulating in Eastern Europe; also detected in America ;

❖ **Two distinct samples**

   ❖ 43 isolates from a global collection between 1982 and 2003

   ❖ 20 isolates, derived from patients at 1 hospital within 7 month

   ❖ Reference strain: TW20

❖ **Platform   Illumina Genome Analyzer GAII**

   ❖genomic DNA preparation   Edge Biosystems Bacterial Genomic DNA Purification kit

   ❖Library preparation   Illumina Indexing standard protocol

   ❖SNP detection   Reads from each isolate were mapped onto the reference genome (TW20)   ssaha_pileup

   ❖Phylogenetic analysis   RaxML v7.0.4
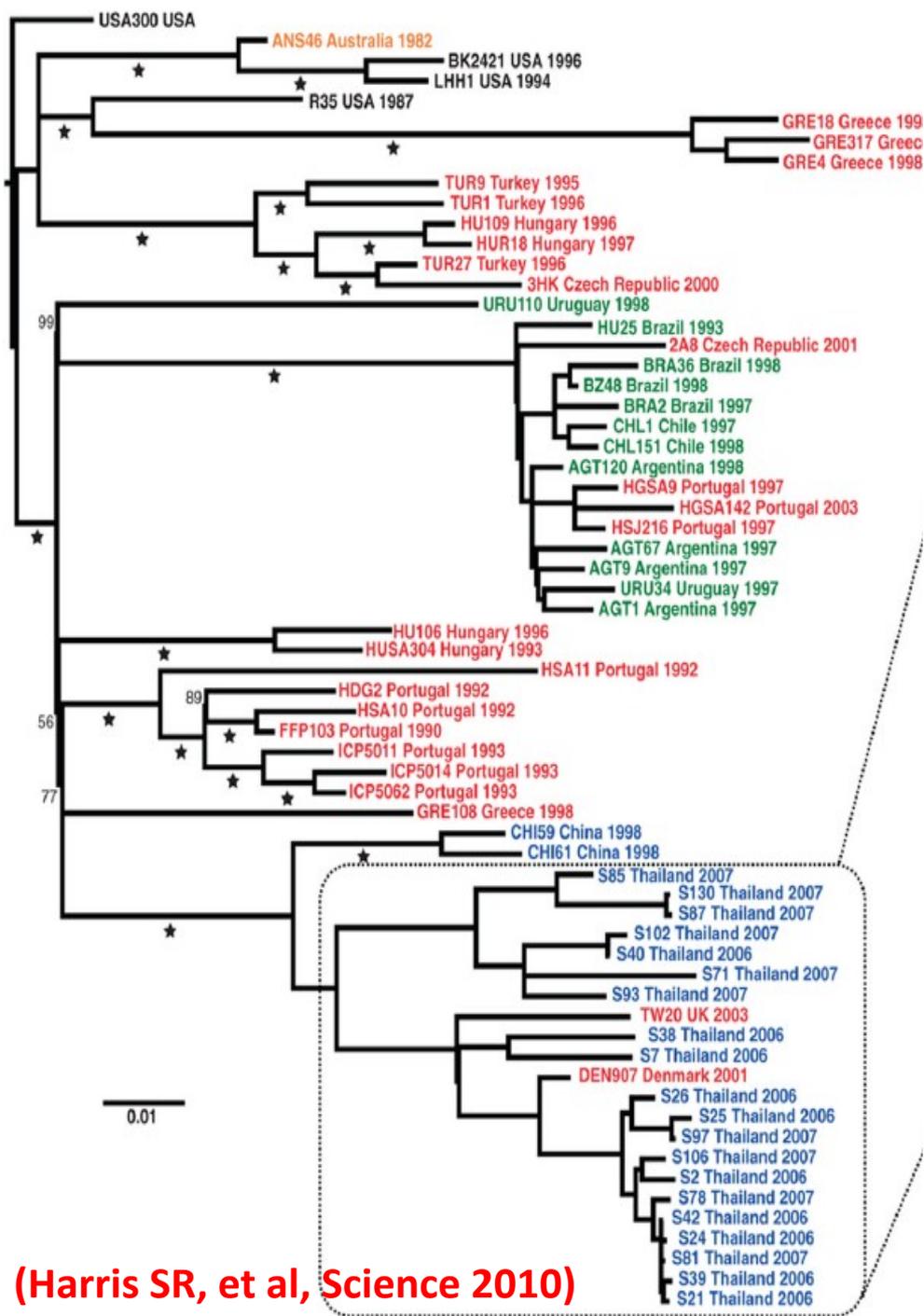
1.Geographical clustering

Maximum likelihood phylogenetic tree (based on 4310 core genome SNPs of ST239 isolates)

The tree showed a striking consistency with geographic source.

Red=Europe
Blue= Asia
Black= North America
Green= South America
Yellow= Australasia

(Harris SR, et al, Science 2010)
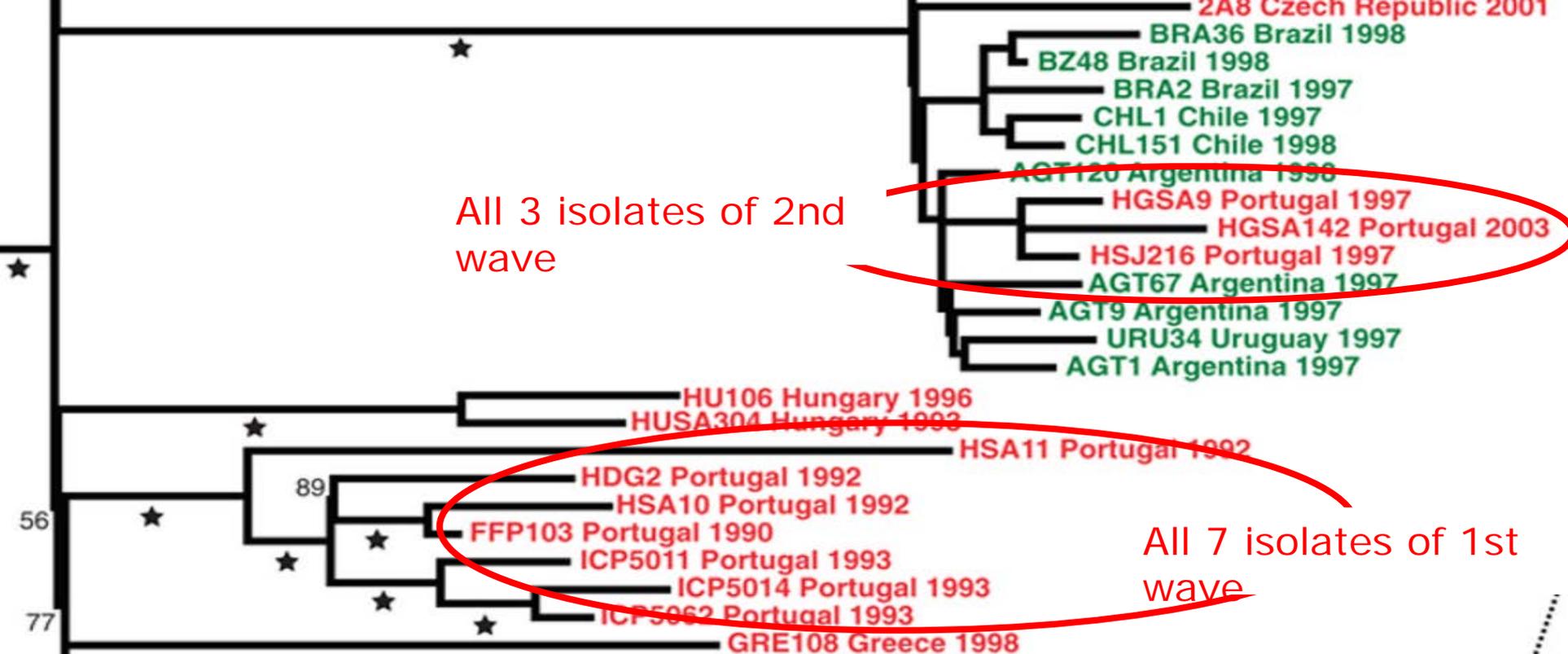
2. **Distinguish possible transmission from endemic infection**

❖ Two waves of ST239 in Portuguese hospitals during the 1990s:

1990-1993;   1997- 2003

❖ Different clusters supporting the **hypothesis** that this second wave in

Portugal resulted from the introduction of a South American variant.
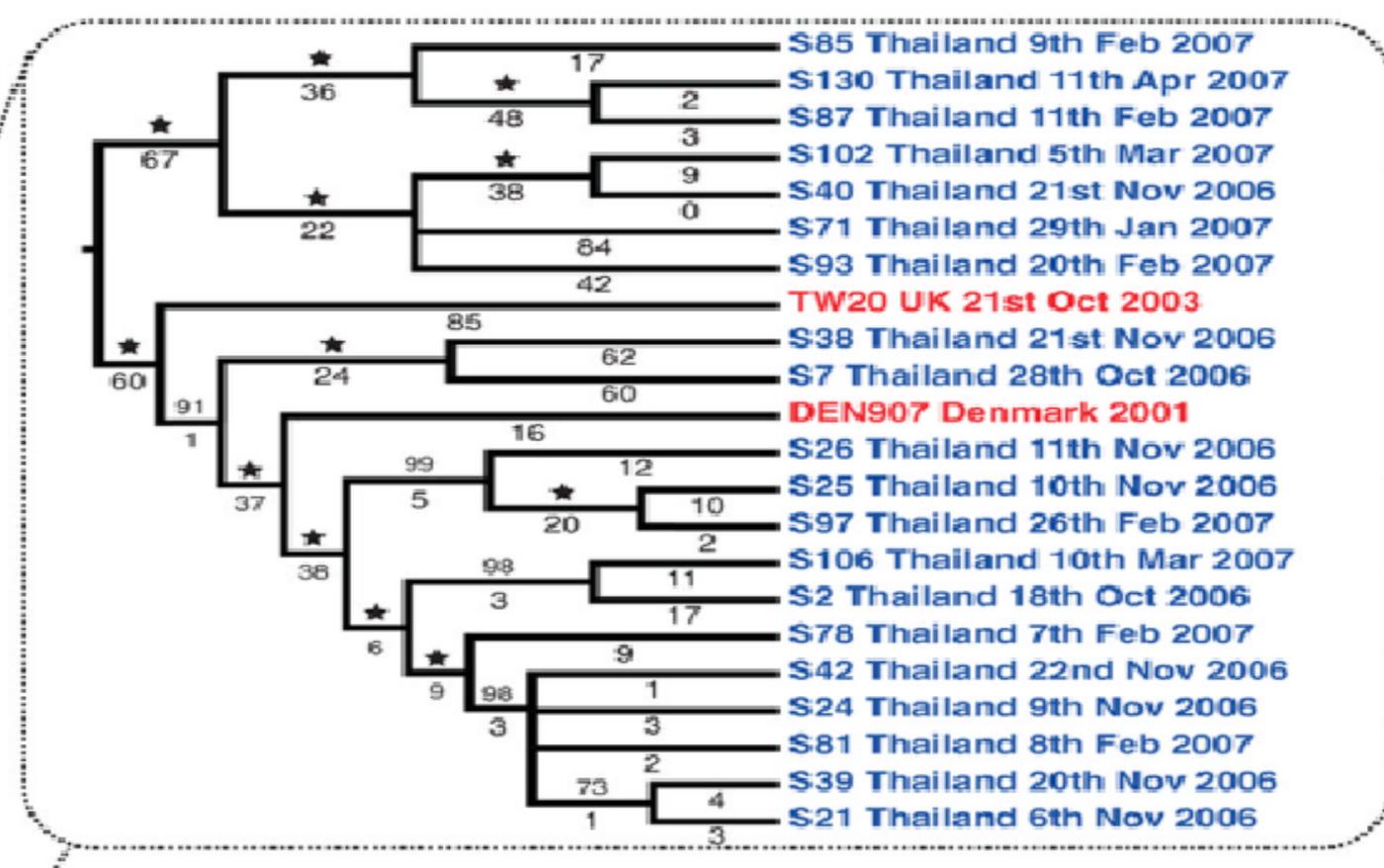
- ❖ 2 European isolates clustered within the Asia clade:
  DEN907, (Denmark,   patient was Thai)
  TW20,   (Ref strain ,from a large outbreak ,London)
  both contain the **core SNPs** of Asia clade : φSPβ-like prophage

- ❖ **potentially points to a single intercontinental transmission event**, most likely from Asia, that sparked the London outbreak.

(Harris SR, et al, Science 2010)

Linear regression of root-to-tip distances against year
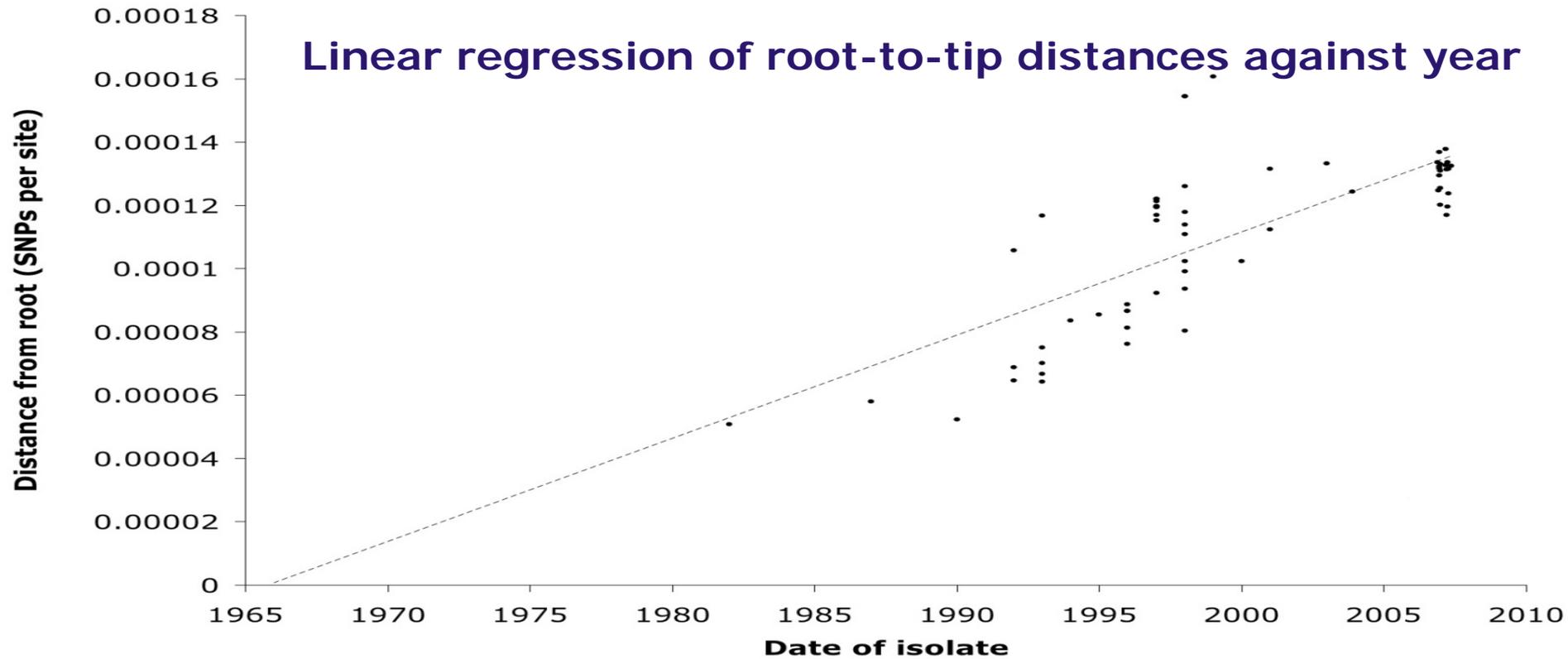
3. Estimates of mutation rate

❖ **The estimated mutation rate**: 3.3 × 10^{-6} per site per year

95% confidence interval (CI) [2.5 × 10^{-6} to 4.0 × 10^{-6}]

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❖ The estimated mutation rate

  3.3×10^{-6} per site per year

  1000 times faster than rate estimate for E. coli

❖ Possible reasons (more evidence needed)

  (1). Greater resolution

     Determine the rate of mutation in the population before

     selection has had time to purify out those harmful

  (2). Reduction in effective population size of MRSA

     Increased accumulation of mutations

Application example 2

Towards an understanding of the evolution of *Staphylococcus aureus* strain USA300 during colonization in community households
(Uhlemann AC, et al, Genome Biol Evol. 2012)

❖ 1. Tracking of interpersonal USA300 transmission

❖ 2. non-synonymous SNP and gene function

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❖ **Target Lineage : USA300 (ST8)**
predominant CA-MRSA strain in US.

❖ **Isolates**

   ❖ 3 clinical and 5 colonizing isolates from 3 unrelated households within 15 month period.

   ❖ Reference strain: FPR3757

❖ **Platform**   **SOLiD 3 System**

  ❖genomic DNA preparation   Qiagen DNAeasy Tissue Kit

  ❖SNP detection   Corona-Lite ; Reads were mapped to the

USA300 FPR3757

  ❖Phylogenetic analysis   Zoom

A

Isolate	1001	2001	3001	1004	2002	1005	1003	1002	HOU-MR	FPR3757
FPR3757	79	75	63	70	113	82	84	605	70	0
HOU-MR	67	63	69	76	101	70	72	601	0	
1002	600	598	602	609	631	601	605	0		
1003	9	65	73	78	101	10	0			
1005	5	63	69	76	97	0				
2002	96	52	100	107	0					
1004	75	73	57	0						
3001	68	66	0							
2001	62	0								
1001	0									

SNPs
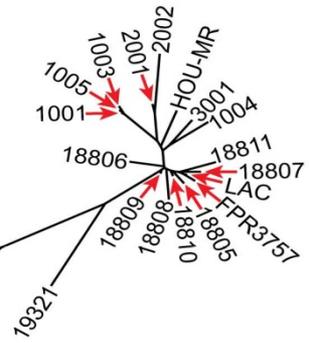
1. Tracking of interpersonal USA300 transmission

While SNP analysis showed limited mutation occurred within 15 months;

The heterogeneity between isolates provide enough discrimination to allow interpersonal Tracking
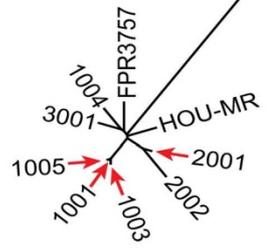
(Uhlemann AC, et al, Genome Biol Evol. 2012)

2. non-synonymous SNP and gene function

Summary of mutations among MRSA isolates

House	Strain	SNPs	IG	NS	S	Ratio
1000	1001	79	31	33	14	2.4 : 1
	1003	84	33	35	15	2.3 : 1
	1004	70	28	28	14	2 : 1
	1005	82	33	34	14	2.4 : 1
2000	2001	75	34	30	11	2.7 : 1
	2002	113	34	37	42	0.9 : 1
3000	3001	63	28	27	8	3.4 : 1

IG=intergenic, NS=non-synonymous SNP, S=synonymous SNP

Although limited mutation occurred ,NS involved in major aspects of MRSA function: adhesion, cell wall biosynthesis, virulence.

Which may contribute to USA300 **fitness and persistence**.   Need further study

(Uhlemann AC, et al, Genome Biol Evol. 2012)

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Benefits and Limitations

❖ Benefits

- ❖ High resolution: single nucleotide differences
- ❖ Allows accurate characterization of transmission events and outbreaks , 'rule in' and 'rule out' links between otherwise indistinguishable isolates
- ❖ Provides a complete inventory of micro evolutionary changes
- ❖ Provides information about the genetic basis of phenotypic characteristics

❖ Limitations

- ❖ Genome assembly challenging
- ❖ Impractical for large population samples
  (Affordability and acceptable turnaround times)
- ❖ A reliable standardized bioinformatics infrastructure needed

Trends

❖ **Technique**

❖Increasing speed ; decreasing cost;
❖unprecedented precision ;
❖batch top available

'It seems that WGS may revolutionize our understanding of MRSA and our ability to manage it as a pathogen.'

❖ **Application**

❖ **Outbreak investigation**
(eg. **Köser CU, et al, N Engl J Med 2012**;
Harris SR, et al, Lancet 2012)

❖ **Micro evolution within special predominant lineage**
(USA 300,ST59,ST772)

❖ **Check reliability of WGS outputs for Phenotypic predictions**
(antibiotic susceptibility, serotype)

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