



Genetic adaptations in hospital-acquired MRSA upon switch from a colonizing to infecting lifestyle in patients developing pneumonia

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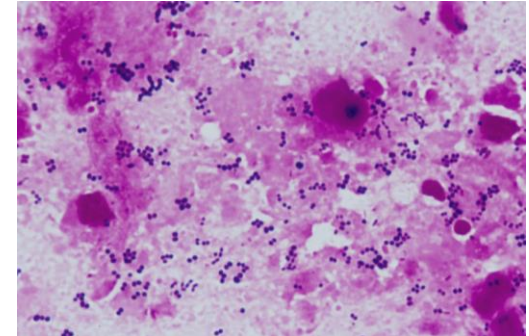
on behalf of SATURN WP1 and 4 study groups

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Genome level identification of MRSA from 5 Serbian patients

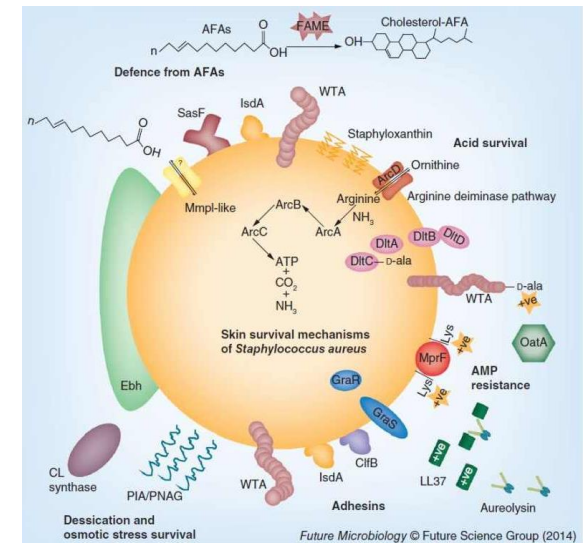
- *Staphylococcus aureus*

- Microbial flora
- Serious pathogen
- Methicillin resistant *S. aureus* (MRSA)



- MRSA infection

- Carriage in anterior nares
- Virulence factors (surface proteins)



EU MRSA acquisition trial

- Impact of combination and sequential antibiotic therapies in selecting resistance
 - Presentation #O381
 - Session: New insights in the control of multi-resistant Gram-negatives
 - Monday, 11 April 2016 - 09:00 - 11:00
 - Hall I



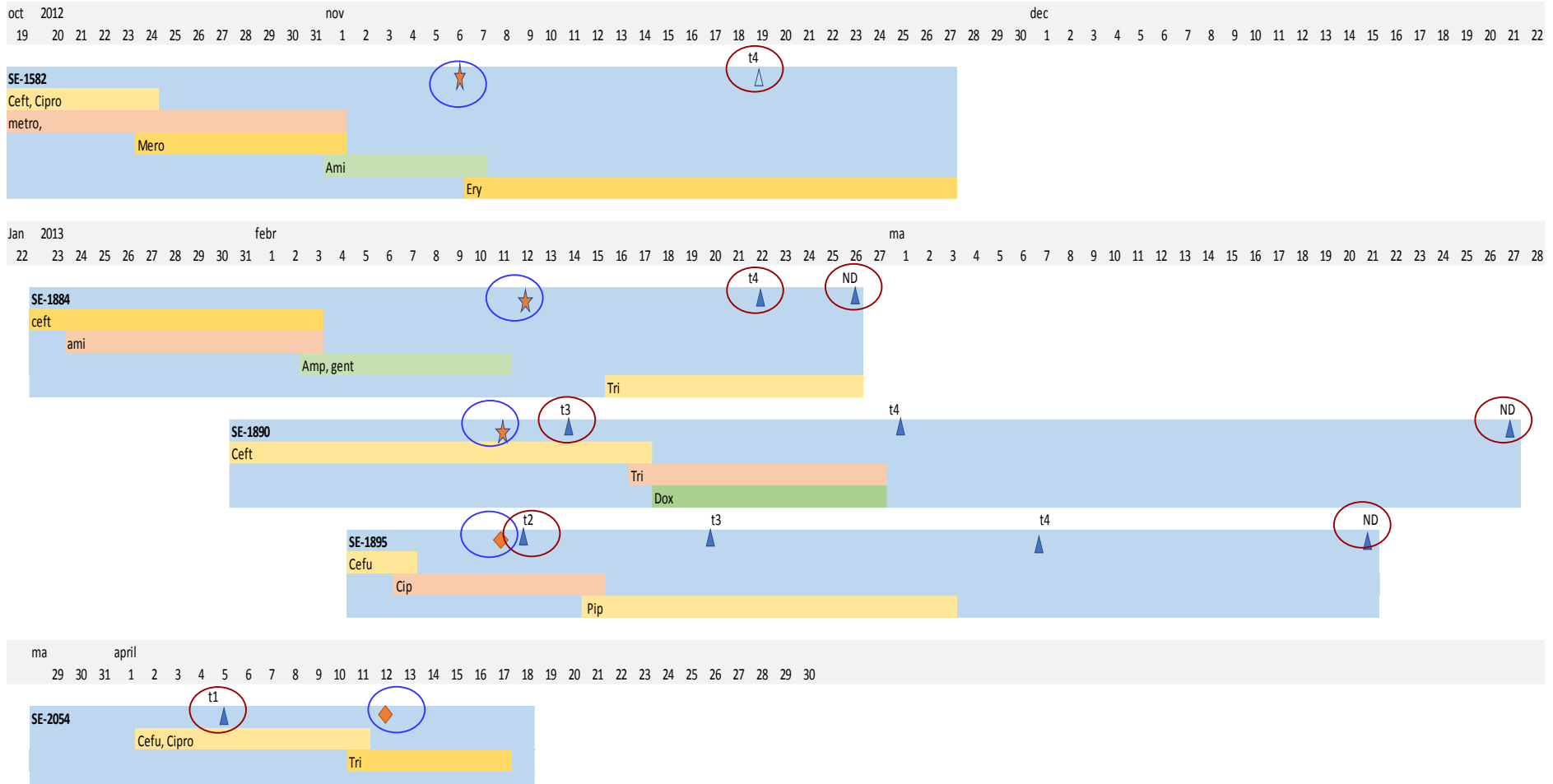
Goals:

- Impact of different antibiotics in hospitalized patients
- Genotypic relation between colonizing and infecting strain in the same patient

Genome level identification of MRSA from 5 Serbian patients

Using AB resistance, MLST, whole genome mapping, WGS

Negative for MRSA at admission



Methods

- Phenotypic characterization
 - Antibiotic susceptibility testing
- Typing methods
 - MLST
 - Whole genome mapping (Opgen Inc)
 - Whole genome sequencing (Miseq, Illumina Inc)
 - Assembly: SPAdes v3.1.0
 - RAST Server <http://rast.nmpdr.org/>
 - Ordering scaffolds: TW20 *S.aureus*
 - SNP analysis: CLC genomics (clcbio, Qiagen)

Antibiotic resistance, MLST & spa results

Patientnumber	Sample type	MLST	CC	Spa type	Antibiotic resistance difference
SE1582	BAL	239	CC239	t037	Doxycycline Mutation: Tetracycline resistance protein TetM:c.784delA
	Nasal swab	239	CC239	t037	
SE1884	BAL	239	CC239	t037	
	Nasal swab	239	CC239	t037	
SE1890	BAL	239	CC239	t037	Doxycycline, Amikacin
	Nasal swab	239	CC239	t037	
SE1895	Sputum	239	CC239	t037	Meropenem, Linezolid
	Nasal swab	239	CC239	t037	
SE2054	Sputum	111	CC5	t2746	Amikacin
	Nasal swab	111	CC5	t2746	

Antibiotic resistance similar for following antibiotics: R: Benzylpenicillin, Ampicillin, Piperacillin, Cefoxitin, Ceftazidime, Ceftriaxone, Cefuroxime, Ciprofloxacin, Gentamicin, Erythromycin, Metronidazole. S: Daptomycin, Trimethoprim-Sulfamethoxazole.

Whole genome mapping (WGM)

WGM is an ordered, high-resolution density whole genome restriction map generated from single DNA molecule

Creating a whole genome map:

DNA
extraction



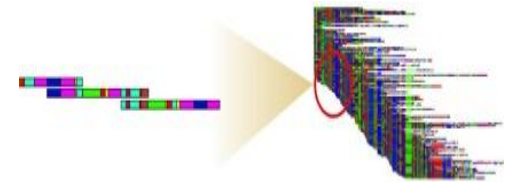
DNA
molecules
immobilized on
flow chip



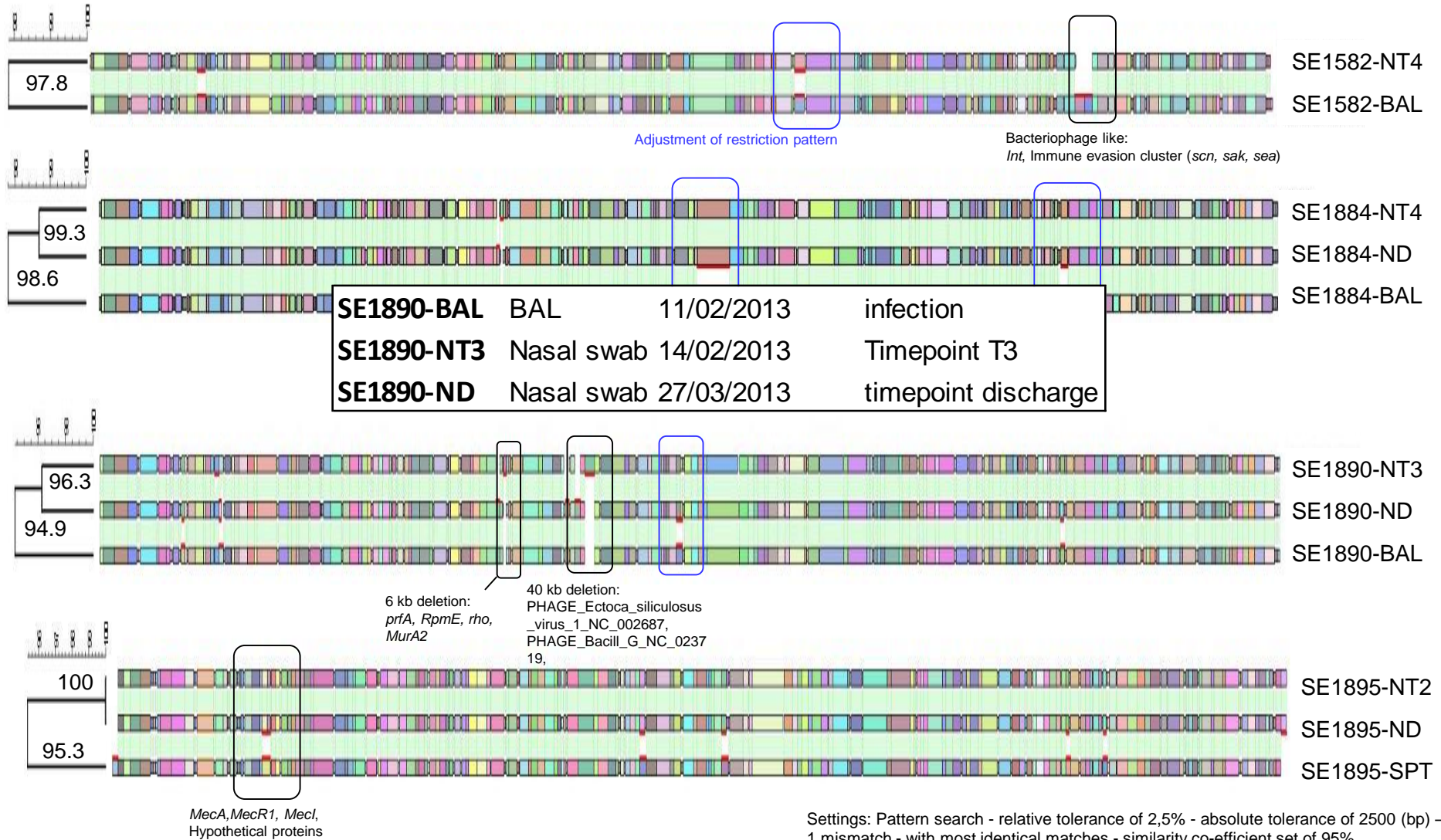
Restriction with
NcoI & RE digest
fluorescent stain



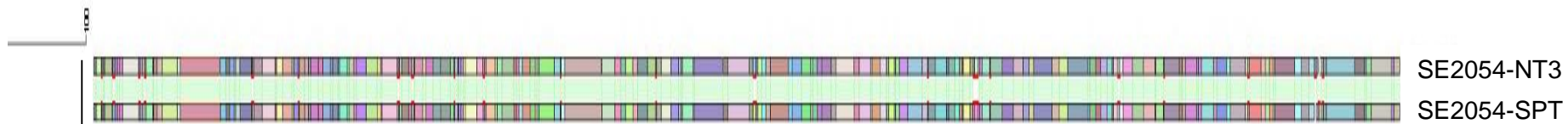
Analysis



Whole genome maps comparison of nasal and infecting ST239 MRSA



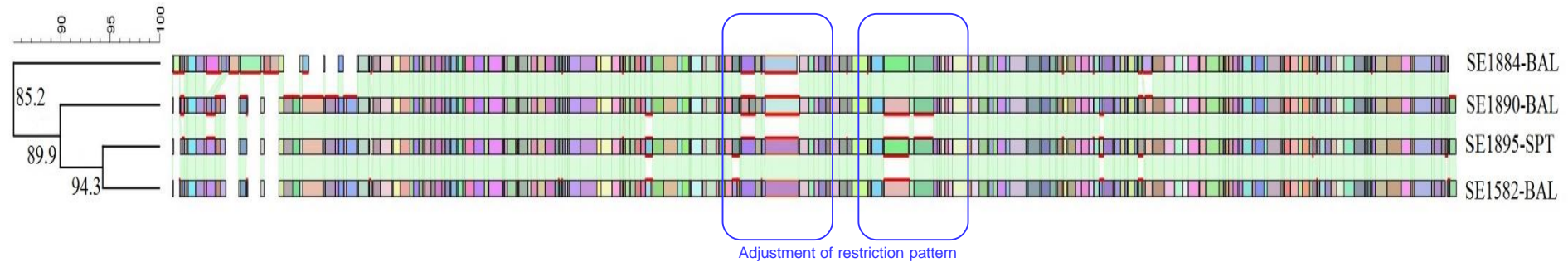
Whole genome maps comparison of nasal and infecting of ST111 MRSA



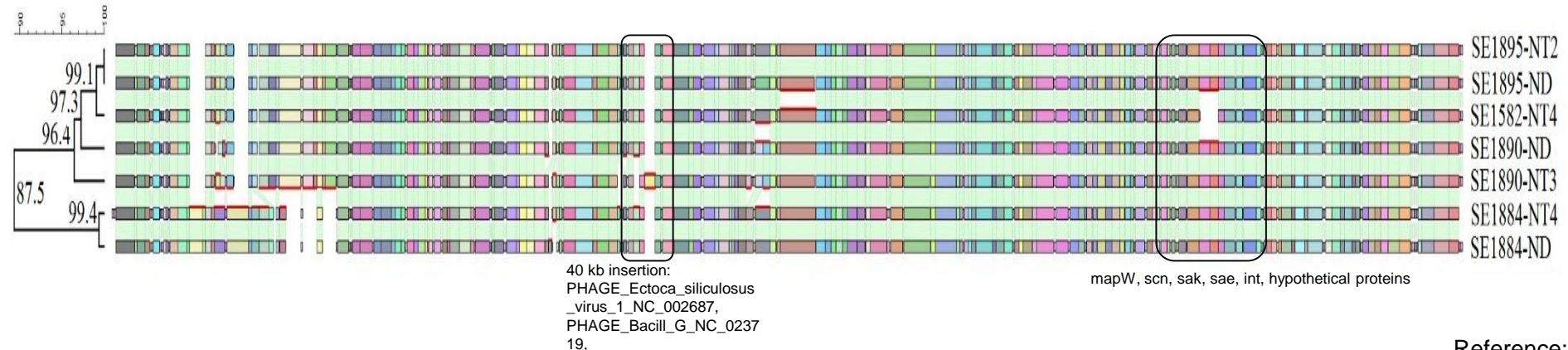
Settings: Pattern search - relative tolerance of 2,5% - absolute tolerance of 2500 (bp)
– 1 mismatch - with most identical matches - similarity co-efficient set of 95%

Whole genome maps of ST239 *S.aureus*

Respiratory strains



Nasal strains



Reference:

TW20 (accession number. FN433596), XN108 (accession number. CP007447.1), TO131, accession number NC_017347) European (Unpublished)

Microevolution of hospital acquired MRSA

	SE1895			SE1890			SE1884			SE1582		SE2054	
	Sputum	Nasal T2	Nasal Discharge	BAL	Nasal T3	Nasal Discharge	BAL	Nasal T4	Nasal Discharge	Nasal T4	BAL	Nasal T1	Sputum
Comparison Whole genome mapping		95.3%	96%		94.9%	94.9%		98.6%	98.6%		97.8%		100%
Whole genome sequencing		99.9%	99.07%		98.44	98.41%		99.34	98.44%		96%		100%
Plasmids Rep20												X	X
Rep21	X	X	X			X				X	X	X	X
Rep7	X	X	X	X	X	X				X	X		
Rep10				X	X						X		
SNPs Non synonymous SNPs		78		2			124				31		

Gene	Function	SNP	Patient	
AraC family	Transcriptional regulator	1549C>T 1673C>N	SE1582 SE1895	Resp vs nasal Resp vs nasal
<i>SasG</i> (<i>LPXTG motif</i>)	Adhesion to desquamated epithelial cells	809A>T, 829T>C, 829T>C 1577A>T, 892A>G 178A>G	SE1895 SE1884 SE1895	Nasal vs nasal Resp vs nasal Nasal vs nasal
<i>SdrD</i>	Adhesin of unknown specificity	3189_3190delTCinsCT	SE1884	Resp vs nasal
<i>Pls</i>		13G>A	SE1890	Nasal vs nasal
	Antiadhesin PIs, binding to squamous nasal epithelial cells:	3122A>C308G>T, 3260G>T, 3132_3134delATTinsGCA, 180_182delATTinsGCA, 170A>C 3189_3190delTCinsCT	SE1895 SE1895	Nasal vs nasal Nasal vs nasal

Young BC et al., Virulence, 2012.
 Corrigan et al., microbiology, 2007
 Foster et al., Nature review, 2014
 Wang et al., Protein & cell, 2013

Conclusions

- The bacteria isolated from nasal swabs and from respiratory infections are closely related
- microevolution is observed
- 2 subclades are formed within one hospital
- Genetic variations were observed:
 - Adhesion
 - plasmids

Funding

- Impact of Specific Antibiotic Therapies on the prevalence of hUman host ResistaNt bacteria, (SATURN, 241796)
- Platform foR European Preparedness Against (Re-)emerging Epidemics, EU-FP7 (PREPARE, 602525)
- European public health initiative EUPHi (Opgen Inc., Gaithersburg, USA)



Thank you for your attention