



University of
Zurich ^{UZH}

Institute of Medical Microbiology



Pathogens

MRSA and UPEC

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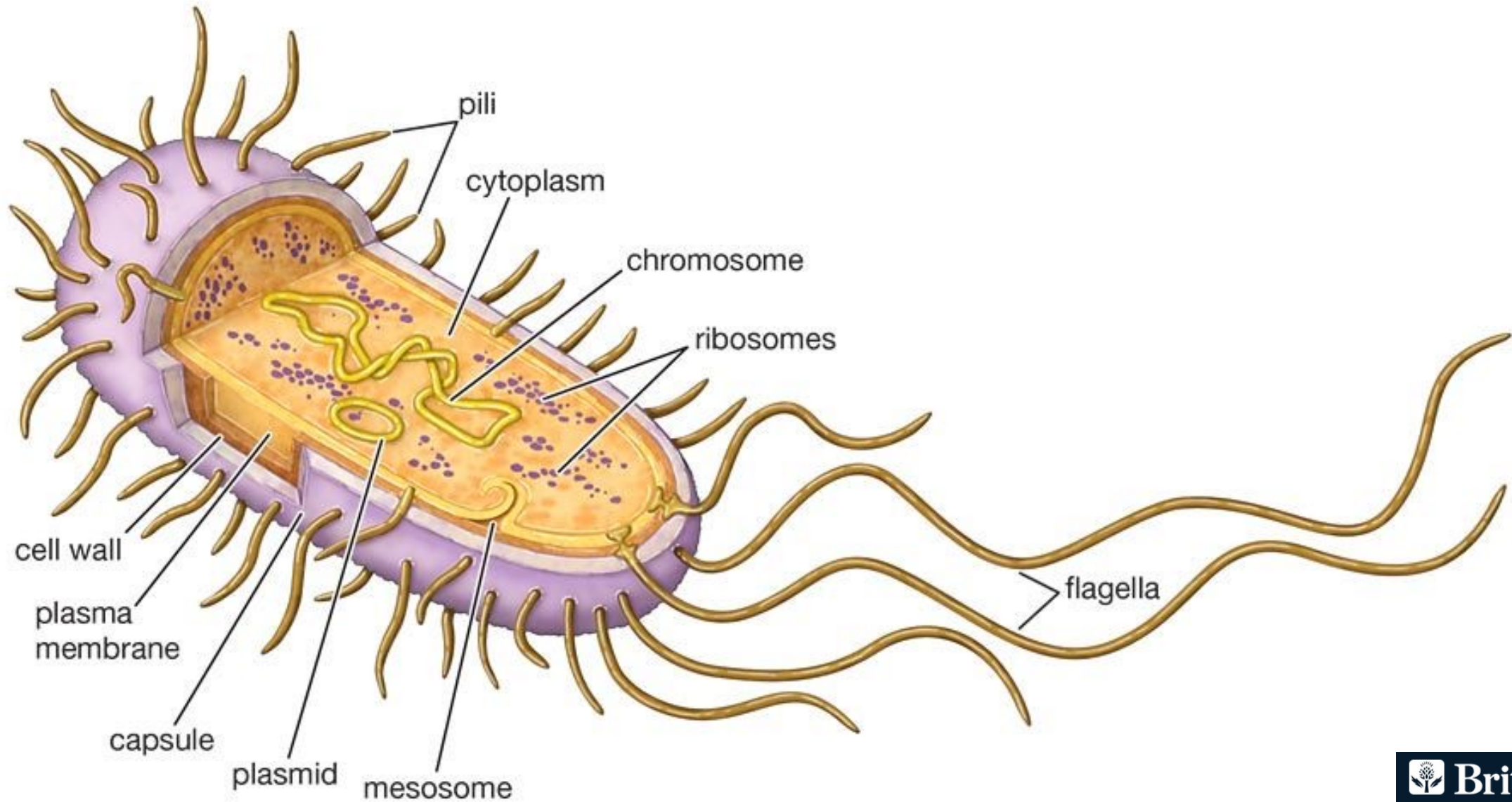
16.03.2023

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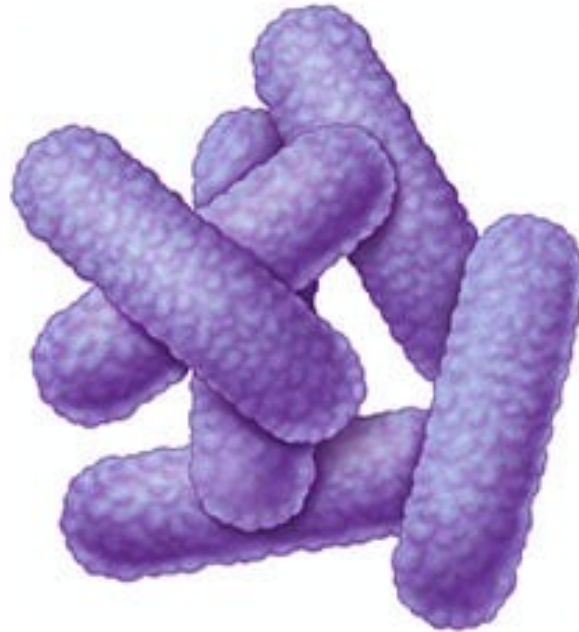
Bacteria I



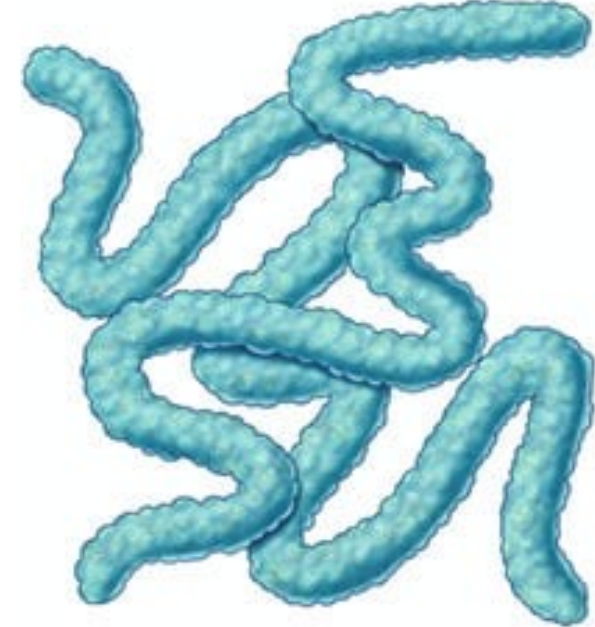
Bacteria II



Sphere-shaped
(cocci)

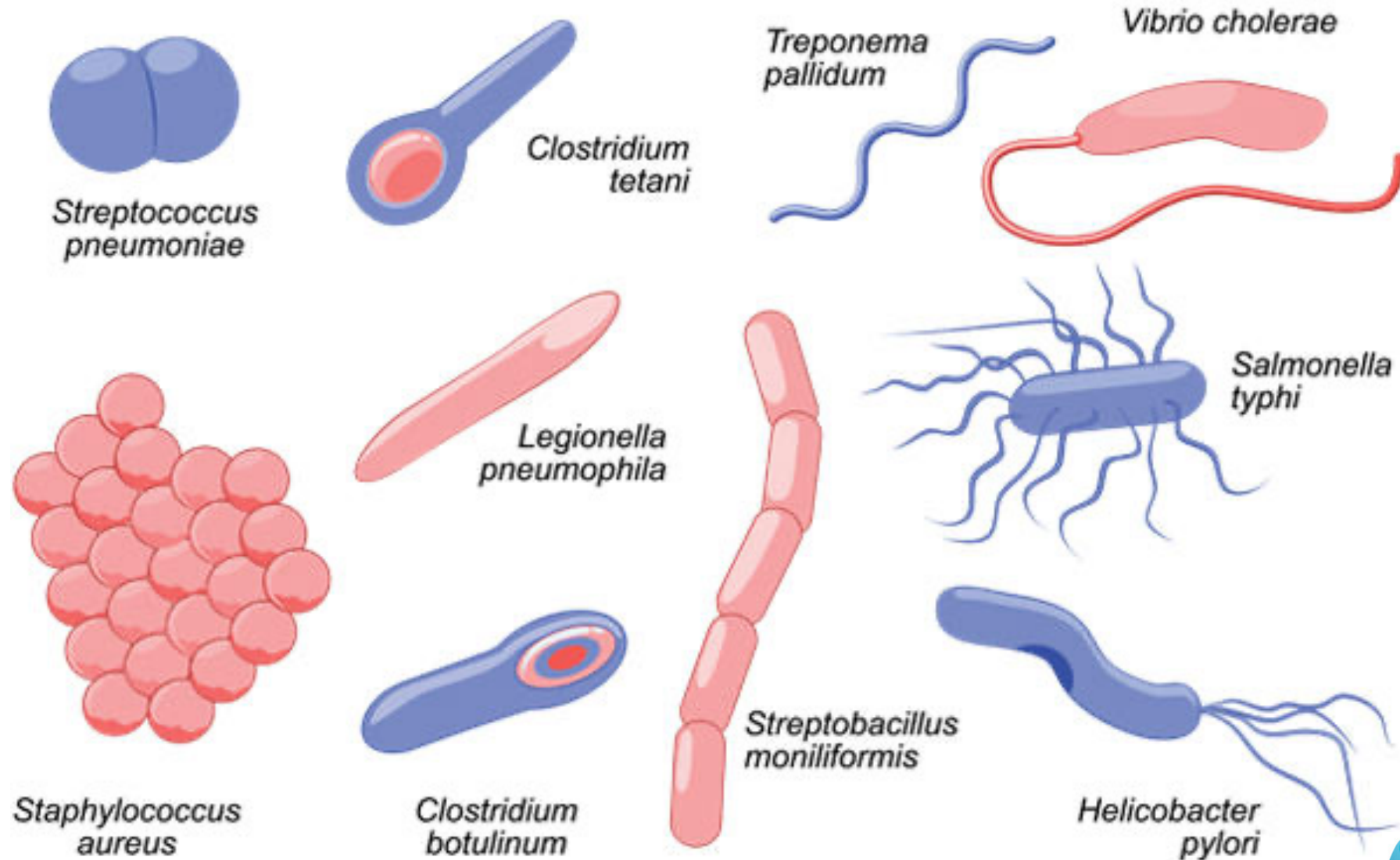


Rod-shaped
(bacilli)



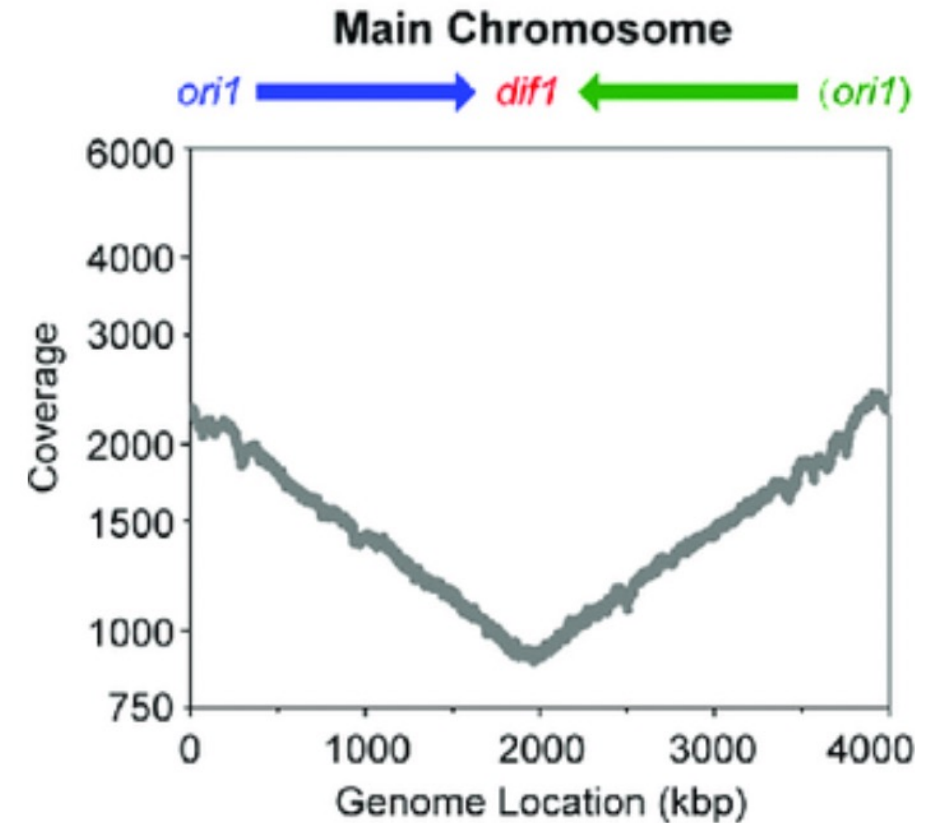
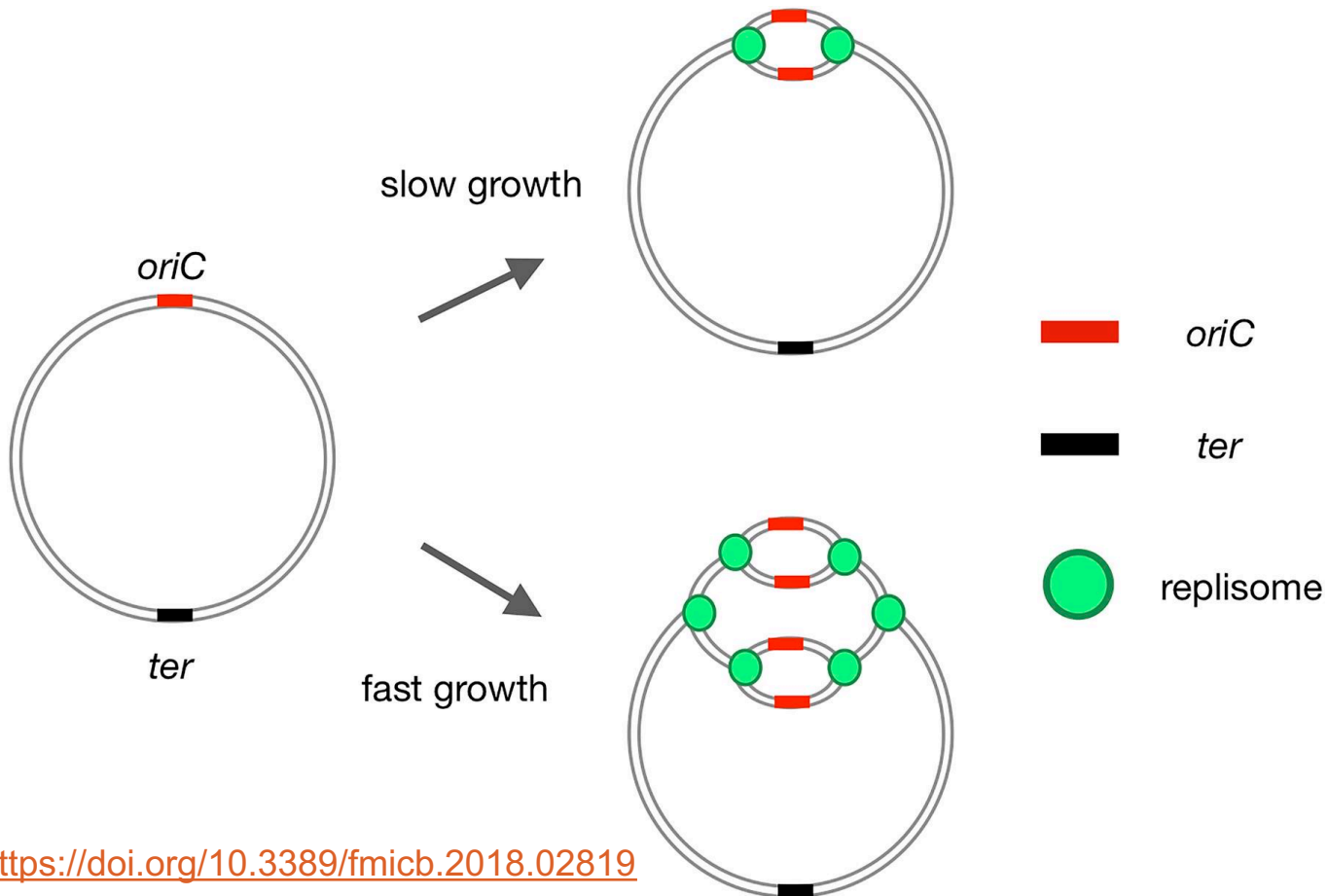
Spiral-shaped
(spirochetes)

Bacteria III



Bacterial genomes I

- “Haploid”
- Can have multiple genome copies per bacterial cell
- The replication mechanism, from origin, to terminus, is reflected in the relative coverage during sequencing

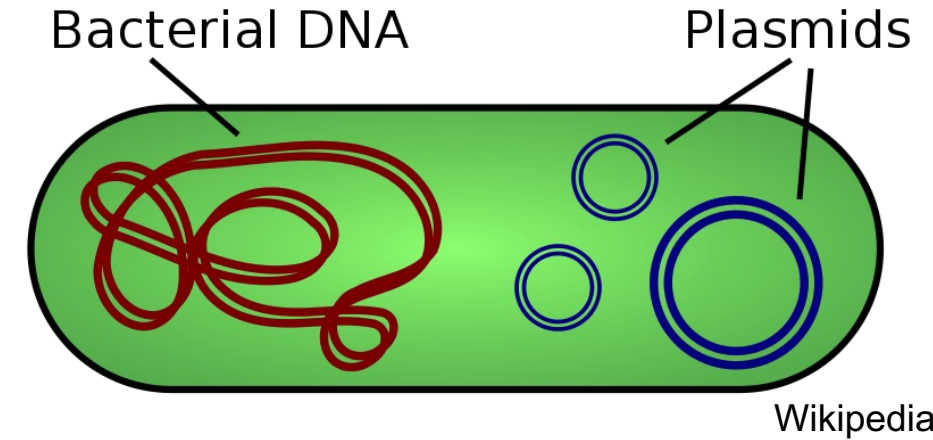
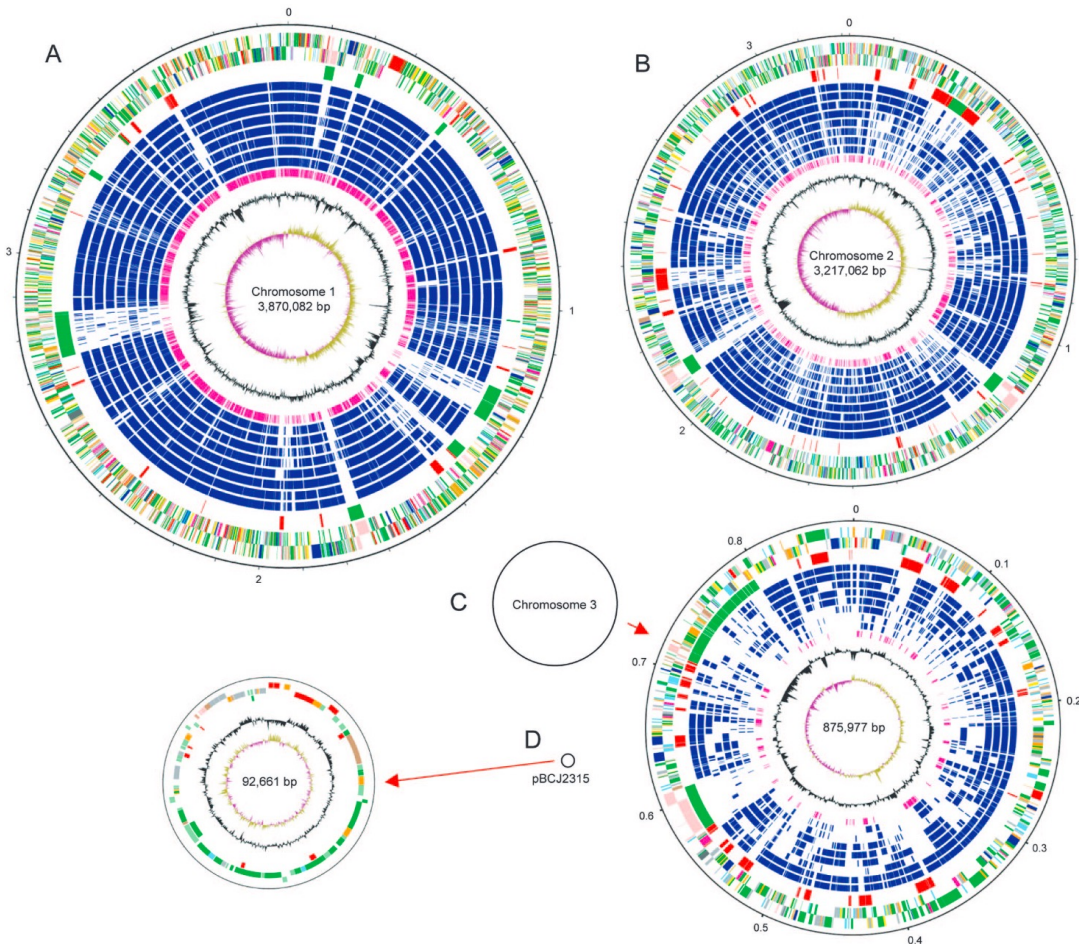


<https://doi.org/10.3389/fmicb.2018.02819>

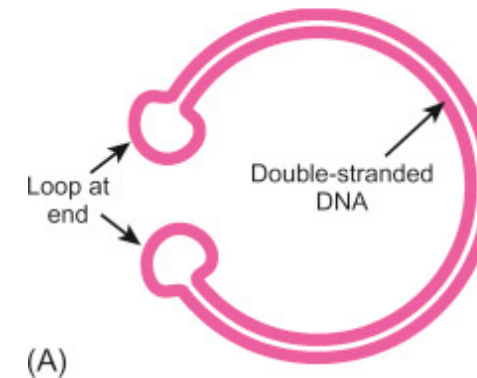
doi.org/10.1128/mBio.02745-20

Bacterial genomes II

- Chromosome (often one circular molecule, sometimes multiple)
- Plasmids (often circular, sometime linear, “accessory” molecules)

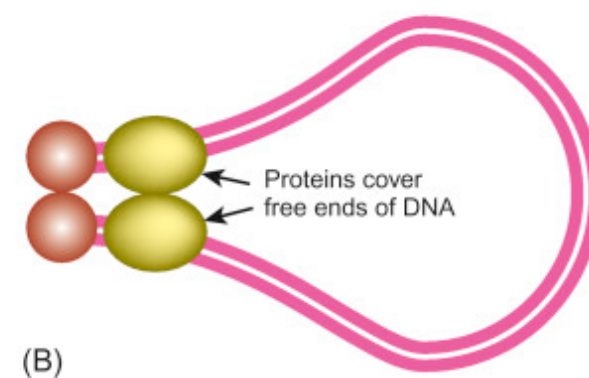


BORRELIA HAIRPIN/LOOP ENDS



(A)

STREPTOMYCES TENNIS RACQUET ENDS



(B)

Burkholderia cenocepacia doi: 10.1128/jb.01230-08

<https://doi.org/10.1016/C2015-0-06229-3>

Pathogens

- A pathogen is defined as an organism causing disease to its host
- Facultative “accidental” pathogens, eg *Neisseria meningitidis*, *Escherichia coli*
- Obligate pathogens, require a host to fulfil their life cycle, eg *Mycobacterium tuberculosis*,

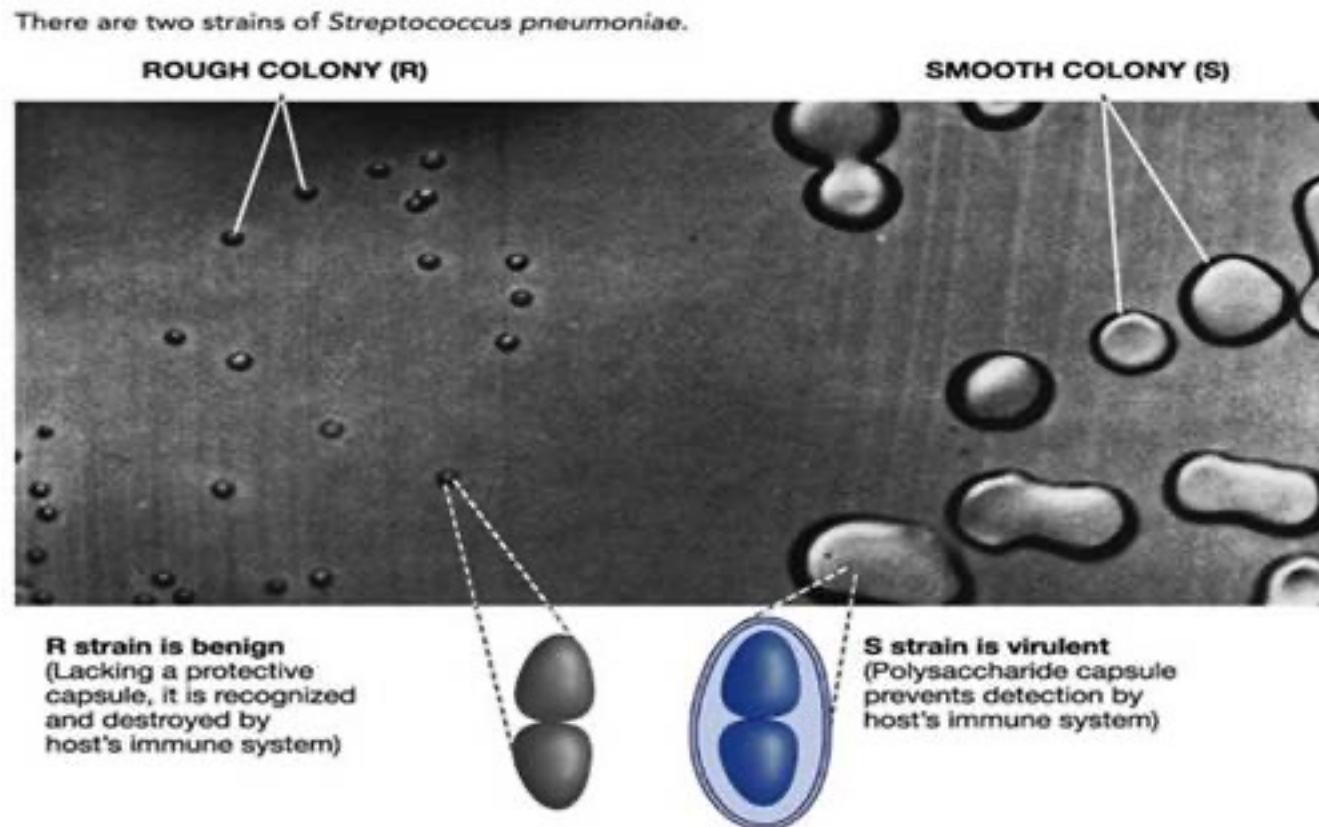
Virulence factors

- Bacterial toxins damage tissues or cells, allowing the pathogen to reach new tissues or exit the cells inside which it replicated
- Host response is immune reaction which can cause further damage and pathogens can benefit
- MORE

A fantastic experiment I

Avery, MacLeod, McCarty, 1944

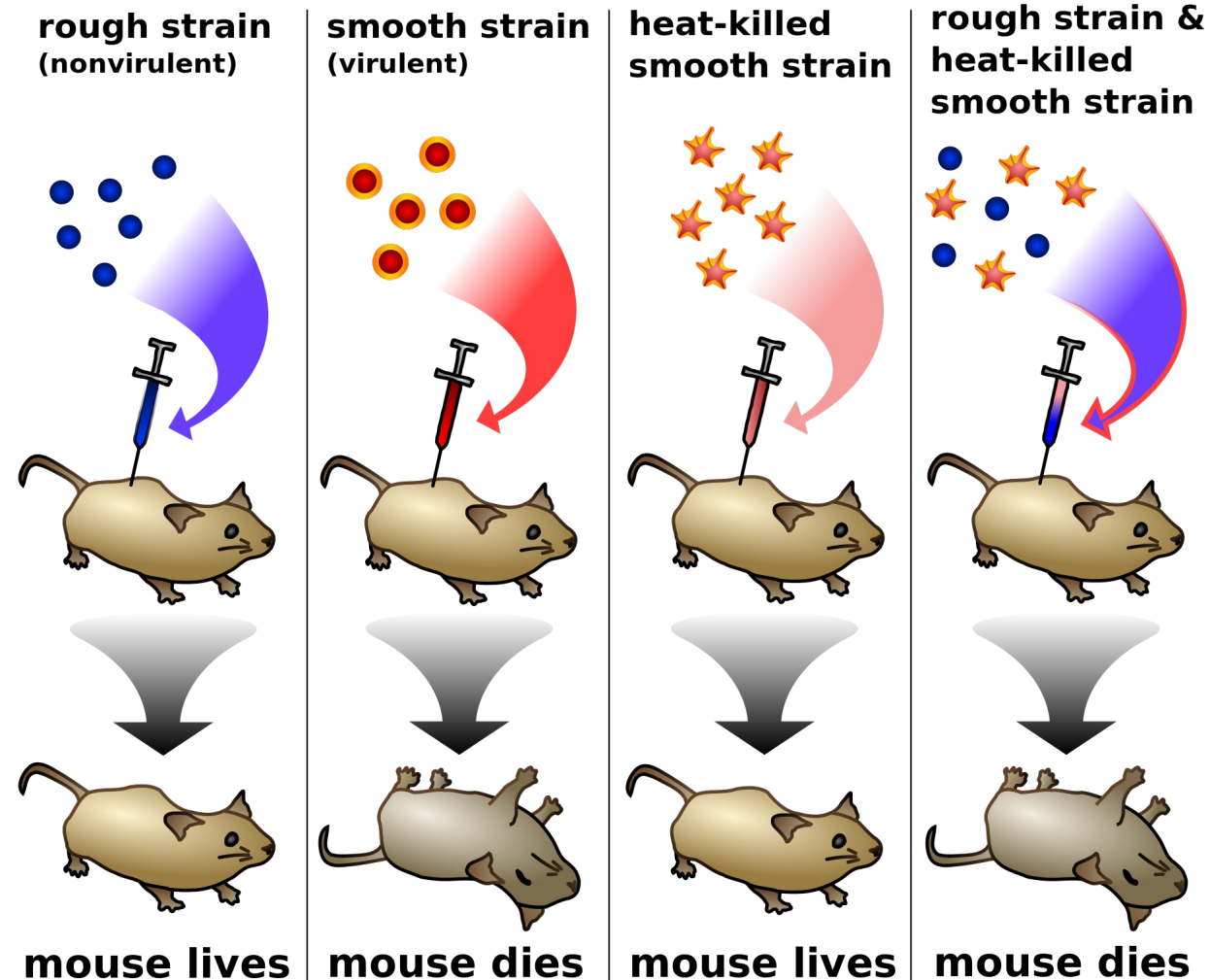
- *Streptococcus pneumoniae* = “pneumococcus”
- On an agar plate, there are “rough” colonies and “smooth” colonies (Capsule- and Capsule+)
- Rough are avirulent in mice, Smooth are virulent



A fantastic experiment II

Avery, MacLeod, McCarty, 1944

– Already observed:



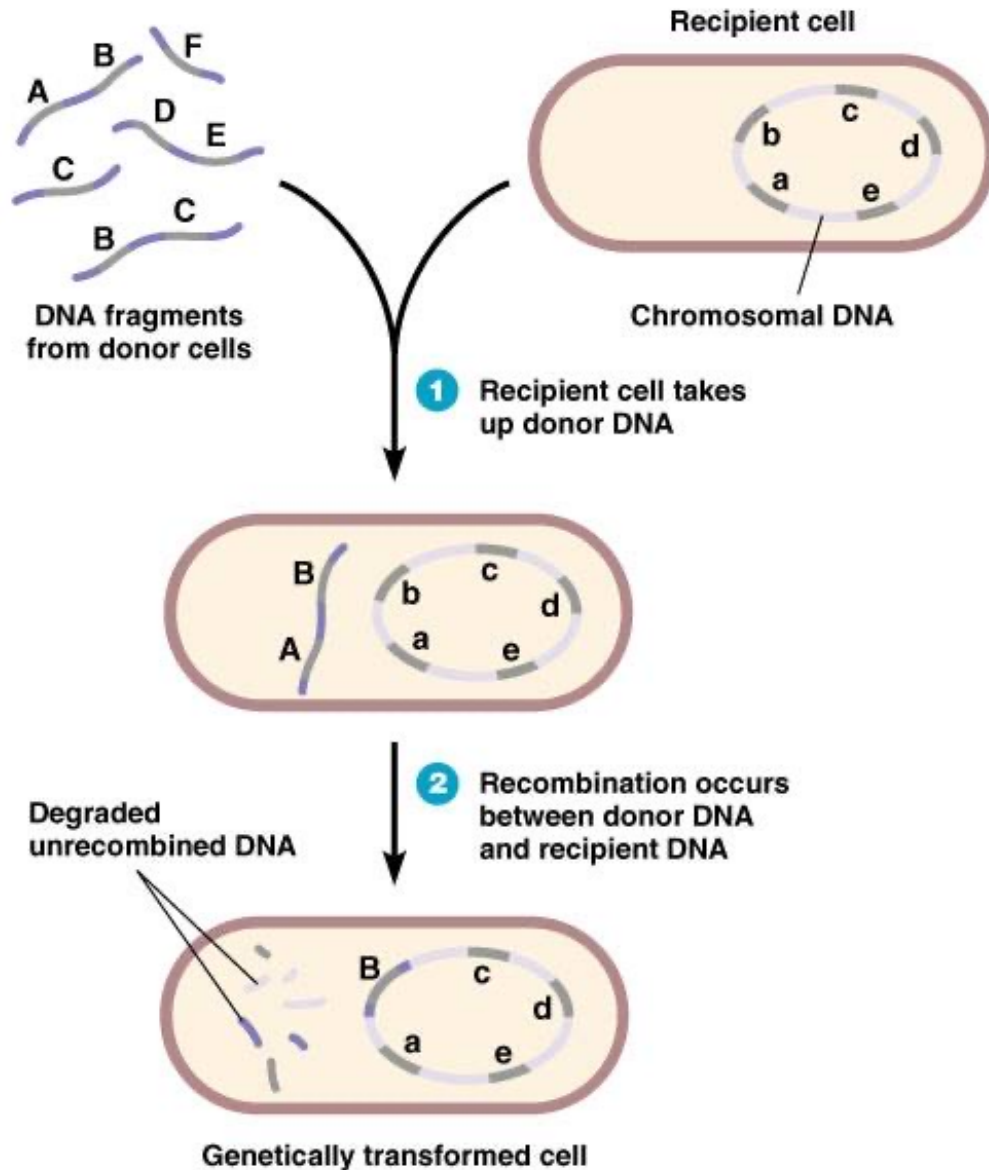
– Question:

– What is the “transforming principle”?

– Using enzymatic methods to digest proteins, RNA or DNA, they found that:

– DNA was the transforming principle

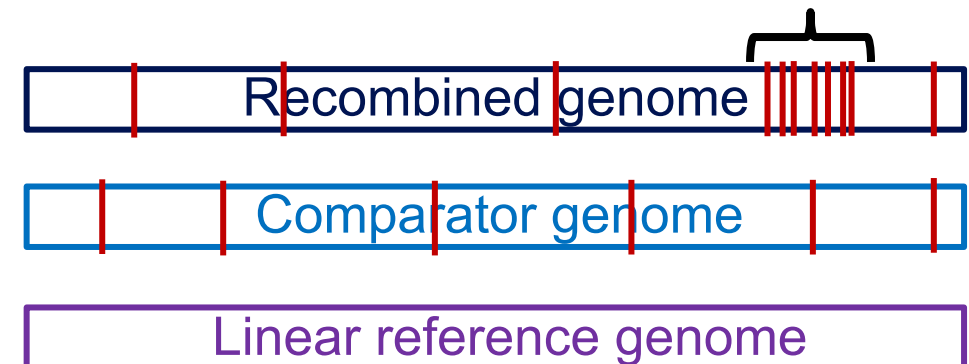
Transformation and recombination



How to identify this is genome sequence:

Mutations (SNPs) often accumulate randomly

High density of SNPs implies gain of “foreign” DNA



A fantastic experiment II

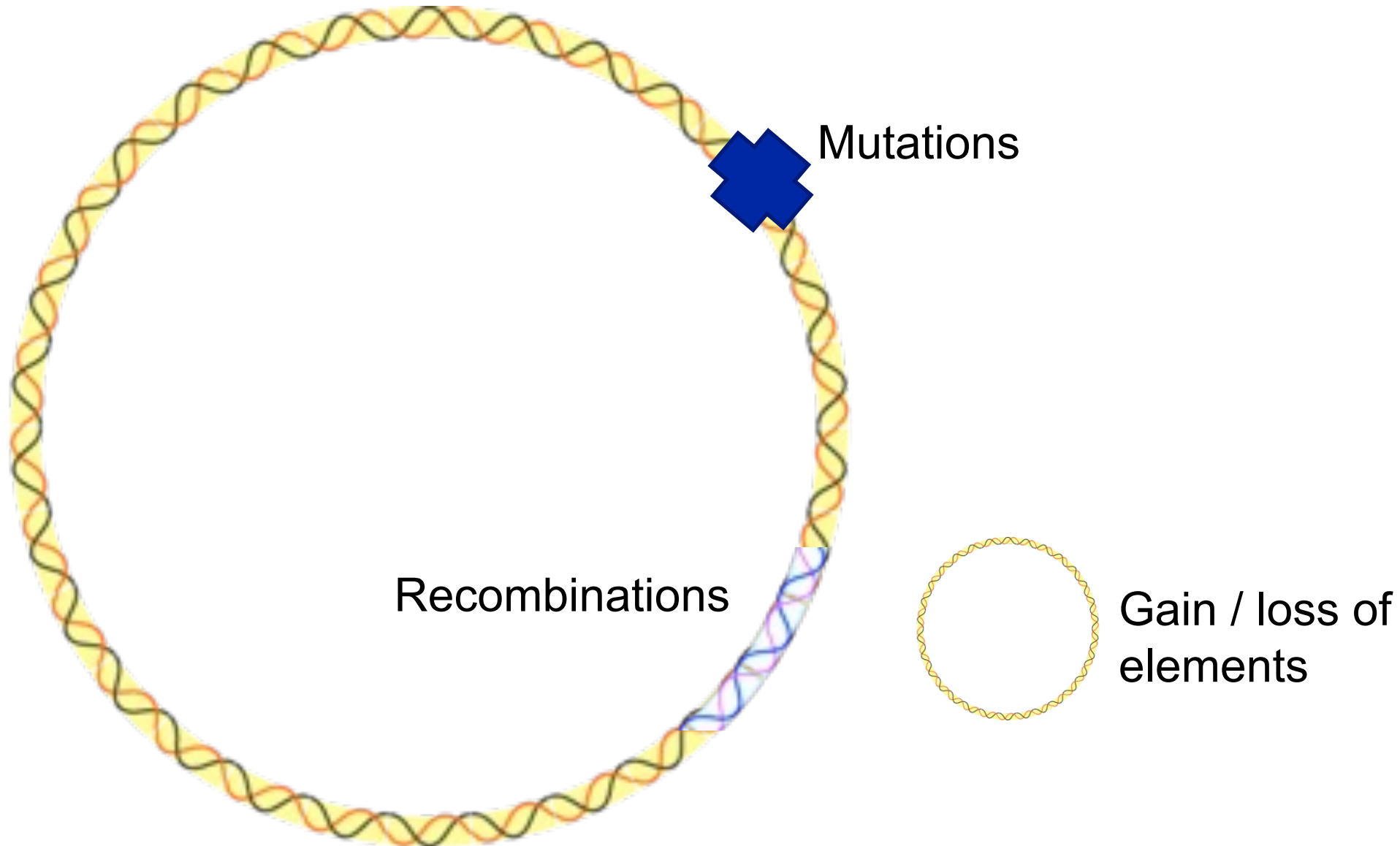
Take home messages

- DNA is the hereditary material
- Bacteria can take up DNA from the surroundings and replace parts of their genome with it
- This “horizontal” gene transfer has very interesting implications
- And is a common feature of many species
- Helping to mix up gene pools

Stories in DNA



Whole Genome Sequencing (WGS) shows genome dynamics



The ESKAPE Pathogens

- Increasing antibiotic resistance
- Multi-drug resistant (MDR)
- WHO priority pathogens
- Commonly healthcare acquired

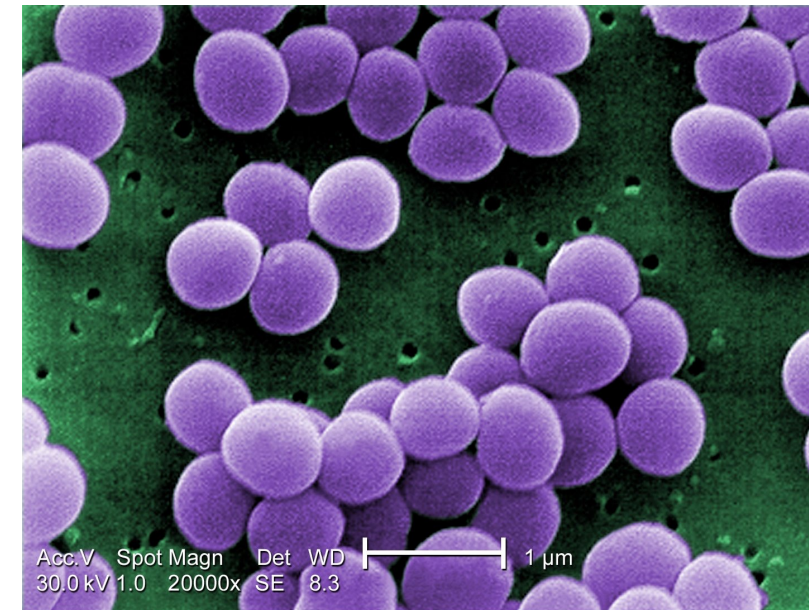


Staphylococcus aureus

- Human commensal = frequently found in healthy people (50%)
- Colonises skin / nose (upper respiratory tract)
- Opportunistic pathogen: can cause abscesses, sinusitis, septicaemia
 - Toxic shock syndrome (TSS) mediated by toxins carried by some strains
 - TSS Toxin (TSST-1) protein encoded by *tst* gene
 - carried on staphylococcal pathogenicity island 1 (mobile genetic element)



Giant Microbes



Wikipedia

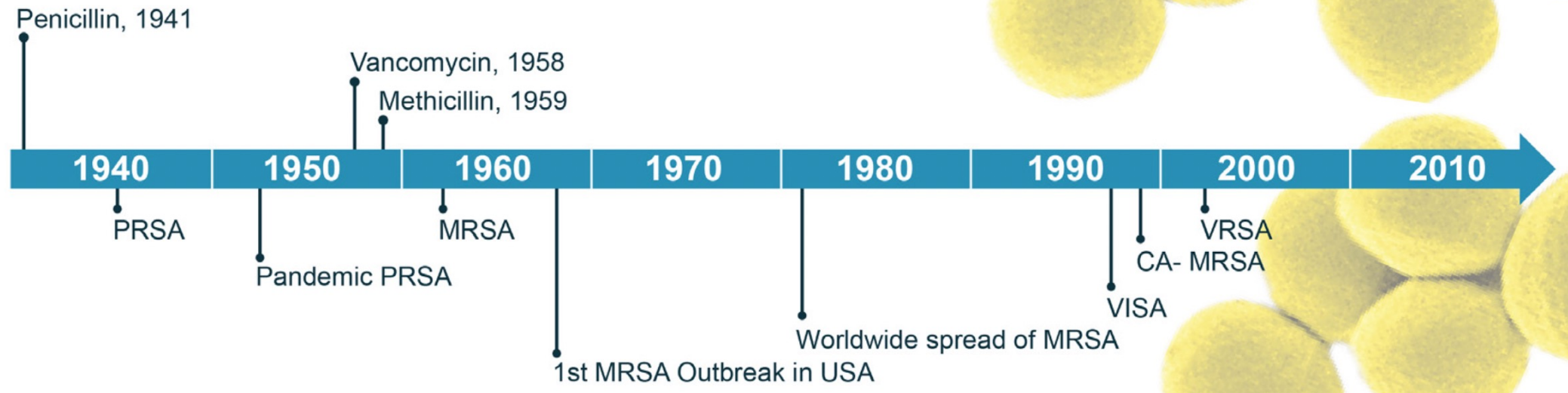
Methicillin resistant *Staphylococcus aureus* (MRSA)

- First case reported in 1961
- Penicillin and methicillin affect *Staphylococcus aureus* cell wall synthesis
- Carries *mecA* which encodes the cell wall transpeptidase protein PBP2A (penicillin-binding protein 2A). PBP2A is not affected by methicillin and penicillin, cell wall synthesis is not inhibited



Giant Microbes

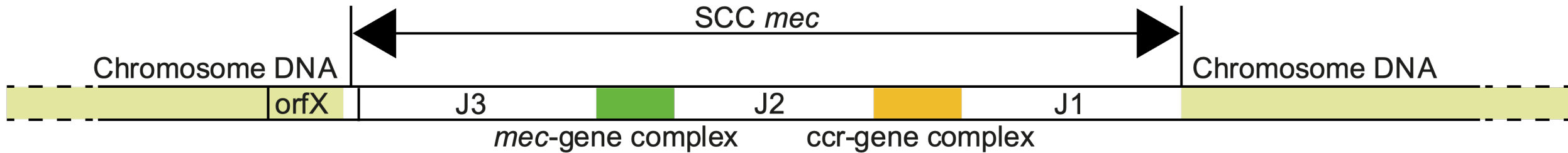
Staphylococcus aureus Drug Resistance and Epidemics



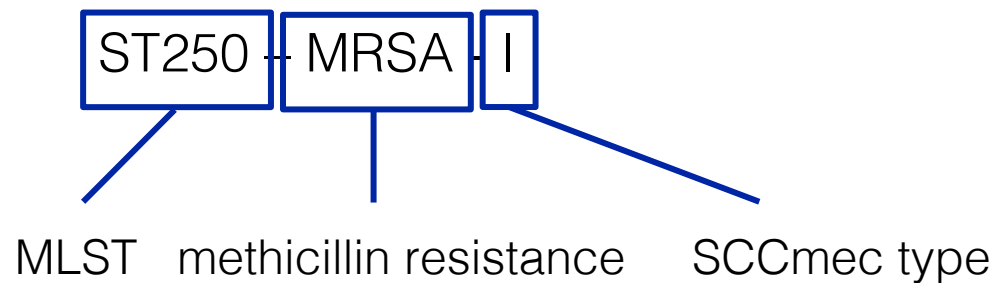
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5482303/>

Resistance spreads through mobile cassette: *SCCmec*

- The *mec* gene is usually carried by the *SCCmec* cassette
- Different cassette types have been identified (I - XII)



- MRSA are categorised (typed) according to a nomenclature established in 2002:

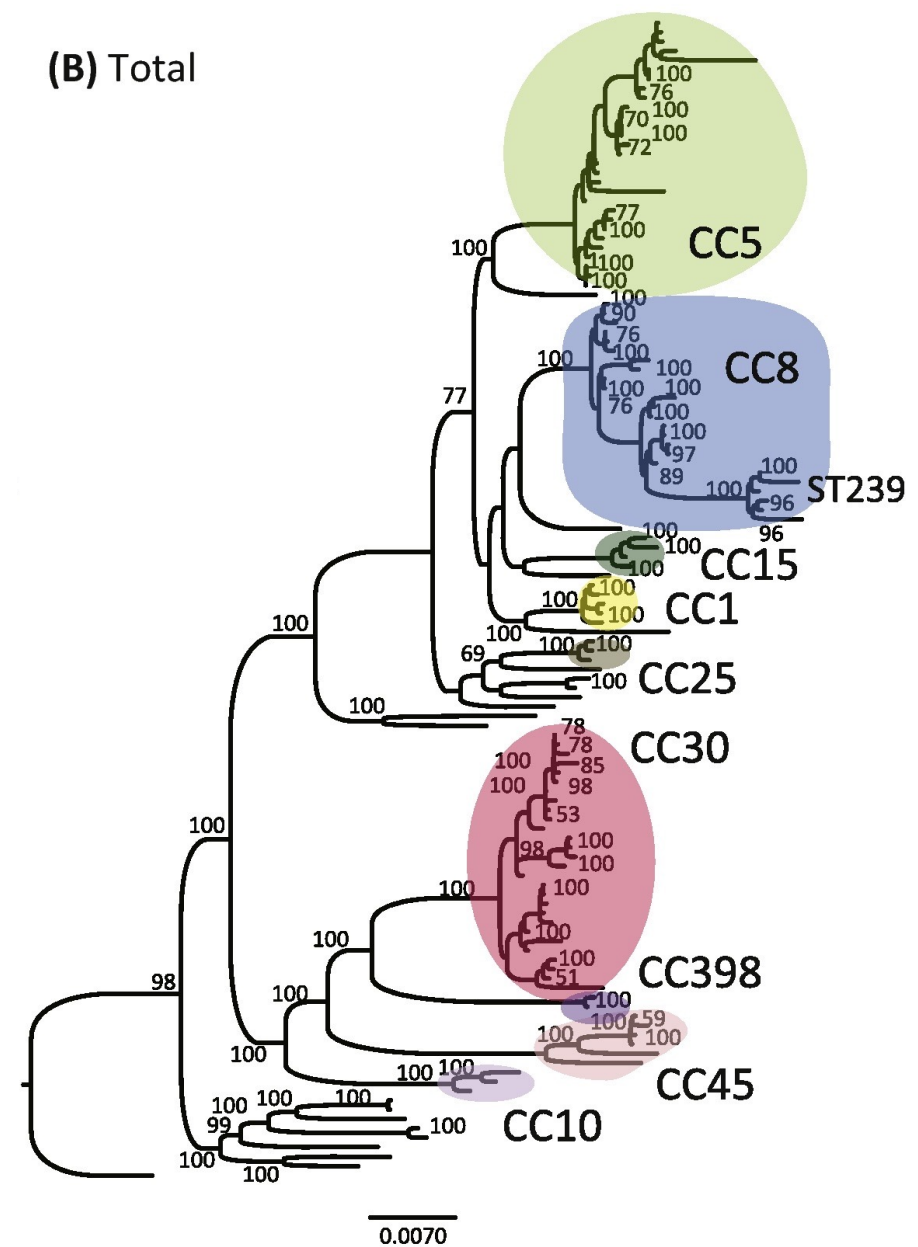


<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5482303/>

Phylogeny of *S. aureus*

- Phylogeny of MRSA and MSSA
- Shape: Discontinuous: Clonal Complexes

(B) Total



<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5482303/>

Comparing older and modern MRSA from Switzerland

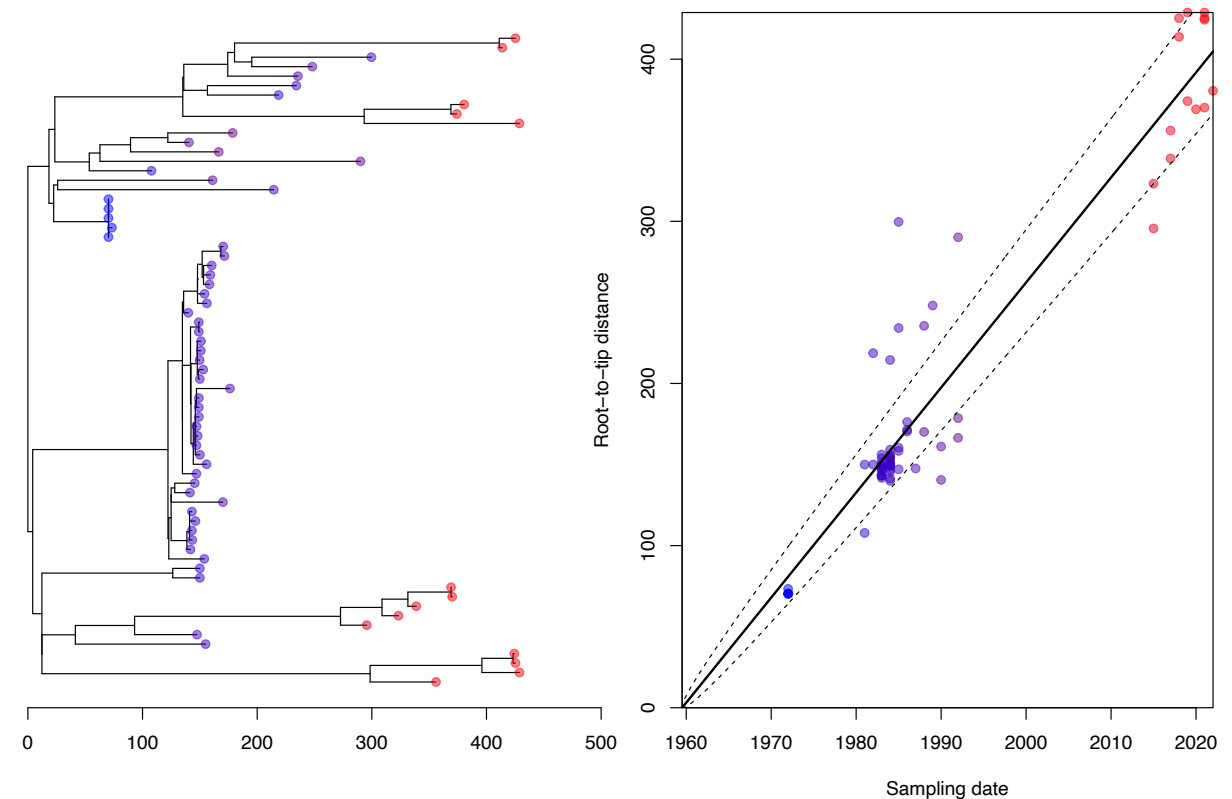
- ST239 and related isolates:
- Phylogeny with metadata
- Largest clade from past not found in present: died out / was outcompeted



BAPS clusters, collection country and collection year mapped to a maximum likelihood whole genome SNP tree of ST239, ST7844, ST368 and ST241 MRSA (n = 69). Leaves colored by sequence type.

- Time tree of same data (Bactdating)
- Correlation of isolation date with number of mutations

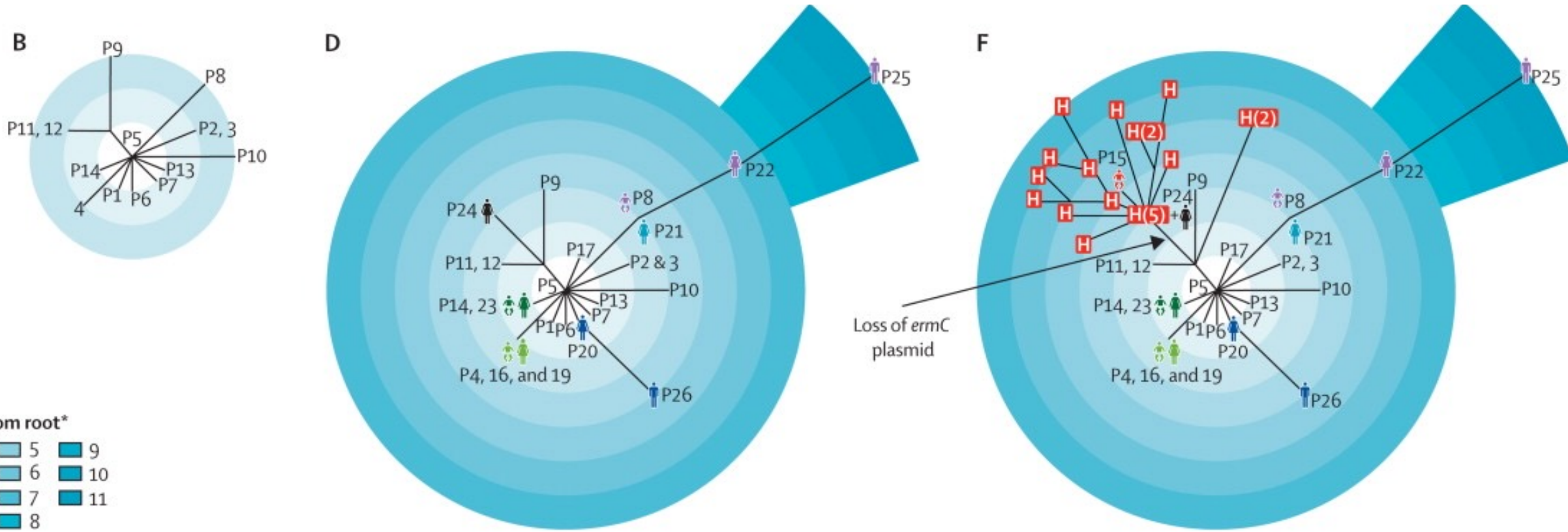
Rate=6.48e+00, MRCA=1959.52, R²=0.90, p<1.00e-04



Maximum likelihood whole genome SNP tree of ST239, ST7844, ST368 and ST241 MRSA (n = 69) adjusted with Bayesian dating of nodes on the left. Root-to-tip distances mapped to sampling date with linear regression shown on the right.

Benvenga et al,
in preparation

Genomic study of MRSA outbreak in a Special Care Baby Unit (SCBU)

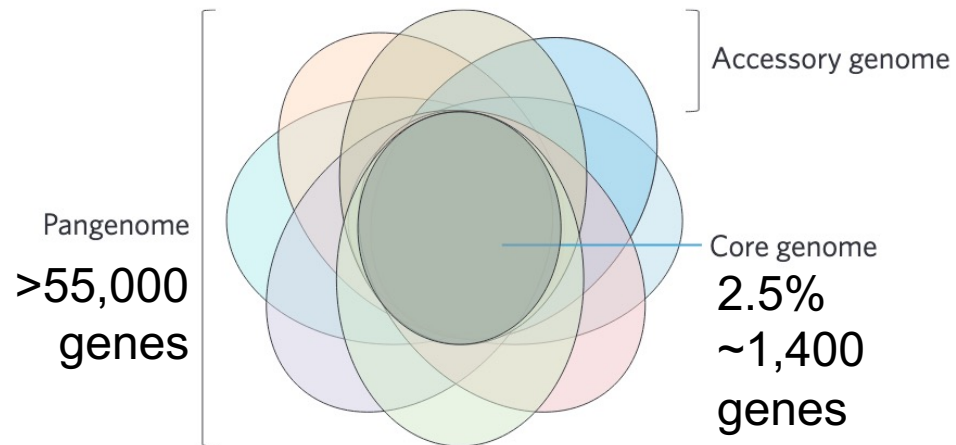


Harris *et al.*, Lancet Infect Dis, 2013

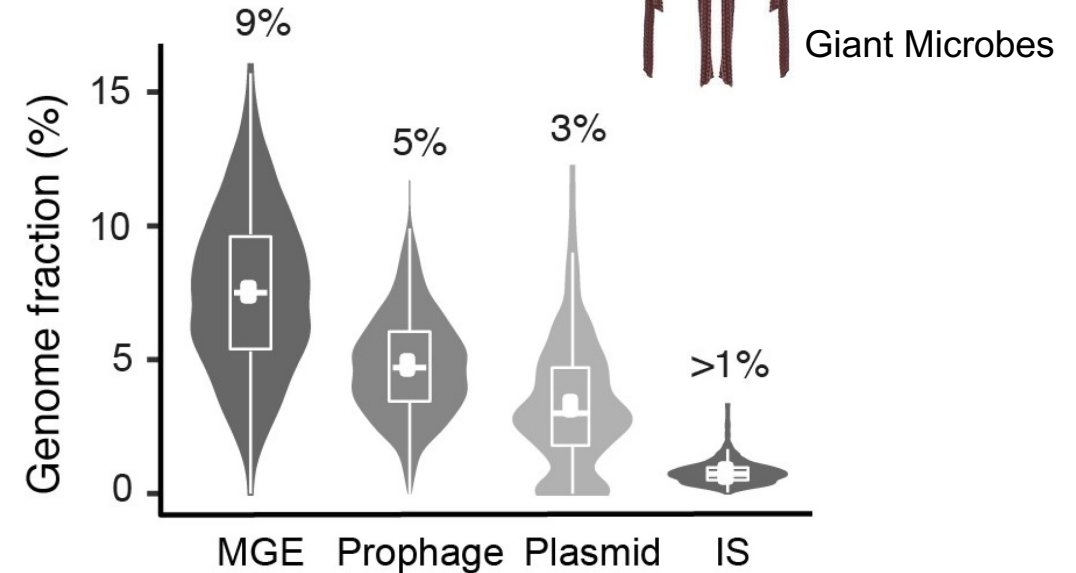
Escherichia coli

– *Escherichia coli* – most frequent cause of UTI

- *E. coli* has a genome of ca 5Mb, 5000 genes
- open pan-genome



- High rates of horizontal gene transfer



- Potential to rapidly adapt
- Differences in gene content between closely related strains
- Varying clinical phenotypes

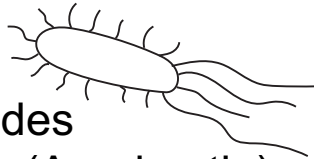
E. coli causing Urinary Tract Infections: Uropathogenic *E. coli* UPEC

- *Escherichia coli* – most frequent cause of UTI
- 65 – 75 % of urinary tract infections (UTI) are caused by uropathogenic *E. coli* (UPEC)
- Most UTI mild symptoms, no long-term effect, some progress to invasive infection (bloodstream infection)

What factors influence the progression of UTI?

Bacterial factors:

- Capsular polysaccharides
- Iron capturing systems (Aerobactin)
- Adhesins
 - Type I fimbriae
 - AfA pili
 - Pyelonephritis associated pili (P-Pili)



Patient factors:

- Age
- Immunosuppression
- Catheter
- Anatomical anomalies
- Co-morbidities

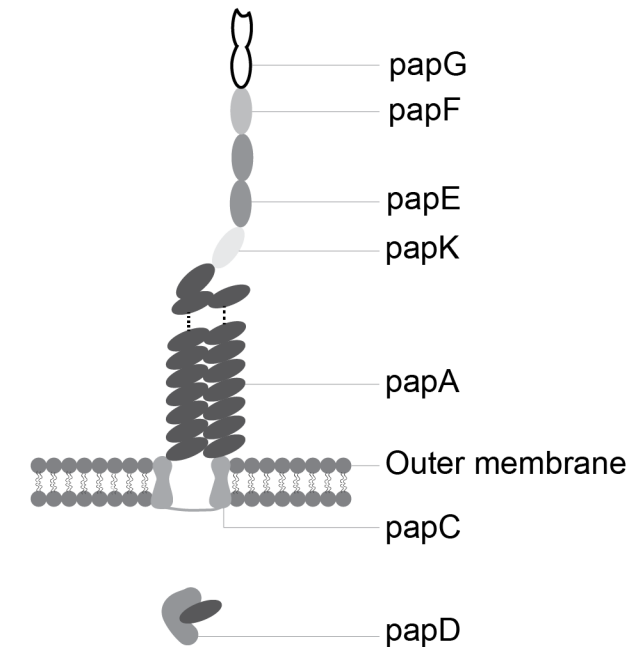
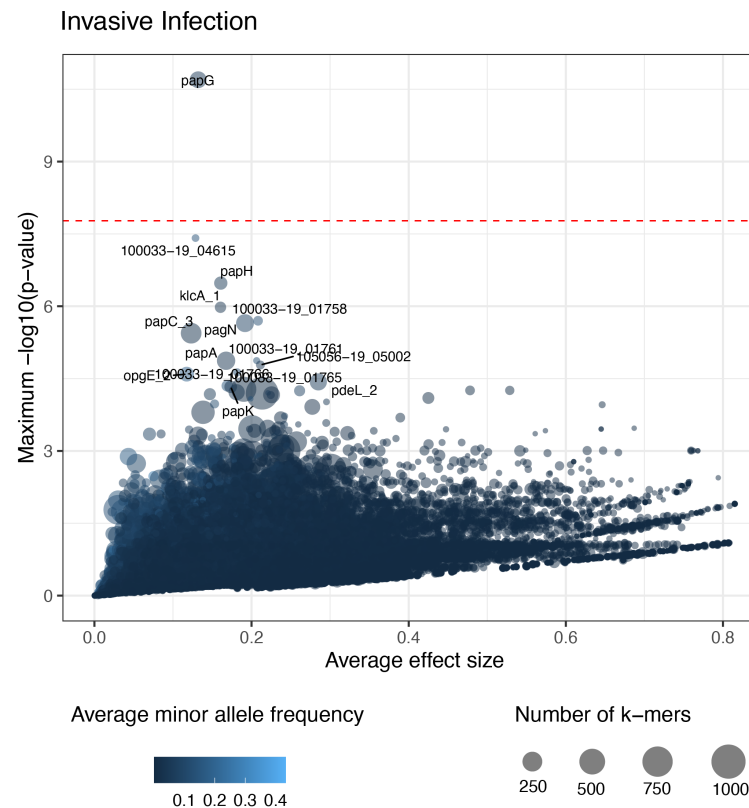


Slide adapted from Aline Cuénod

Study on UPEC



- 1079 isolates collected from 831 clinical cases
- Whole genome sequenced
- Known UPEC lineages: enrichment of UPEC phylogroups
- Bacterial Genome Wide association study, identifying factors (genes) associated with specific phenotypes, identified *papGII* as involved in UPEC progression to invasive infection
- PapGII is one version (allele) of the adhesive tip of the PAP pilus



Cuénod et al,
in preparation

Summary and Learning Goals

This course will cover the following topics:

In this practical course, you will learn about whole genome sequencing (WGS), which provides the ultimate resolution for bacterial comparisons (typing), showing relationships between isolates and the presence of genes encoding antimicrobial resistance and virulence. For clinical applications, WGS of pathogens isolated from patients is the state of the art to detect transmissions, outbreaks, and to optimise antibiotic treatments. The faster the information is available, the better. This course will teach hands-on real-time nanopore sequencing, compare nanopore data with validated Illumina data, and show participants how to perform comparative genomic analysis, as well as resistance and virulence determinant detection. Methods within wet and dry labs will be compared and discussed, the influence on clinical decision making will be explored, as well as how WGS data can feed back into diagnostics.

With the aim of generating these learning outcomes:

Hands-on experience with whole genome sequencing using Oxford Nanopore Technologies methods.

Comparison with Illumina data: theory and practise.

Bioinformatic analysis of resulting data by core genome, phylogenetics and kmer methods, using online tools and open source softwares.

Critical comparison of varied analyses.

Interpretation of results in relevant/clinical context.

Relevance of virulence factors and development of diagnostic assay

Assessment

- **We will assess these through presentations, written work, and interaction during the course:**
 - Presentation skills, spoken and preparation 40%
 - Writing skills: accurate, succinct 40%
 - Interactions with peers, teammates and tutors 10%
 - Engagement and interest 10%

The results of your course assessment will be communicated to you approximately one month after the end of the course

Course material



https://gitlab.uzh.ch/appliedmicrobiologyresearch/Blockkurs_MicrobialBioinfo

Reading suggestions

Basic Microbiology

<https://microbiologysociety.org/why-microbiology-matters/what-is-microbiology/bacteria.html>

<https://www.britannica.com/science/bacteria>

Pathogens

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5648414/>

Avery MacLeod and McCarty

https://en.wikipedia.org/wiki/Avery%E2%80%93MacLeod%E2%80%93McCarty_experiment