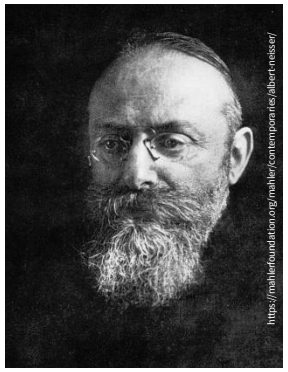
Gonococci – a local view on a global pathogen

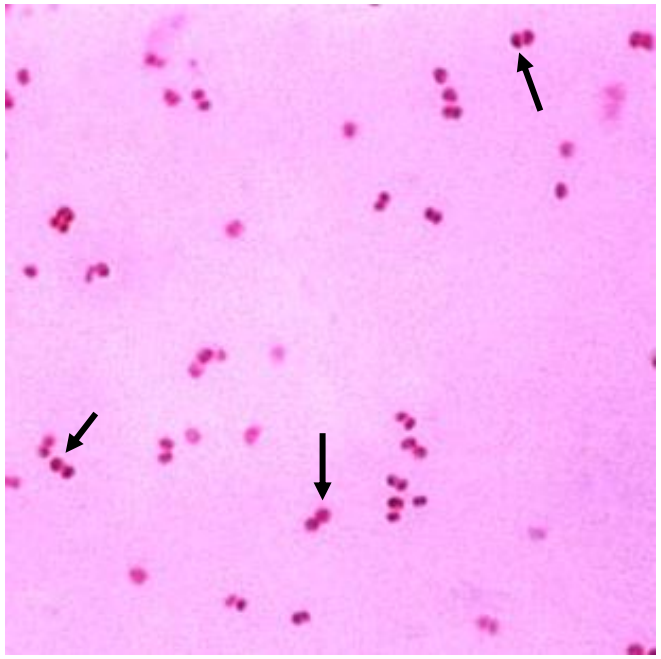
Molecular Diagnostics Symposium Zurich 27.02.2025

Dr. Frank Imkamp

https://www.istockphoto.com/photos/gonococci

Neisseria gonorrhoeae





→ Discovered by 1879 **Albert Neisser** ("*Micrococcus*")

→ Obligate human pathogen

→ causative agent of **gonorrhoea**; 2nd most common **sexually transmitted disease** worldwide (No. 1: *Chlamydia trachomatis*)

→ Aerobic, facultative anerobic

→ Gram negative **diplococci**

→ Very **susceptible** to drying, temperature fluctuations (pre-analytics!!)

→ **Complex growth requirements** (modified Thayer-Martin agar), CO_2, 35 – 37 °C

→ Genome size: ~2.2 Mbp (~2'600 genes), conjugative plasmids, cryptic plasmid

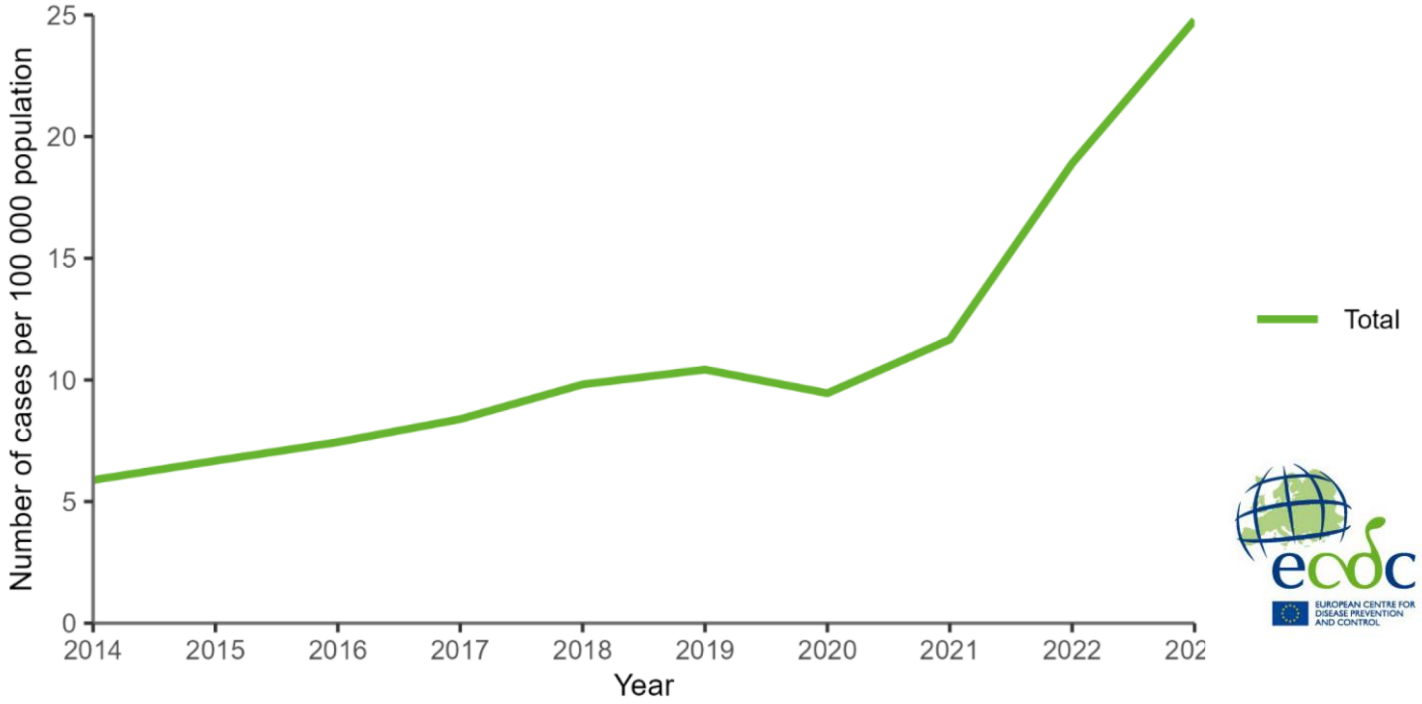
Epidemiology - global

82.4 million people were newly infected with gonorrhoeae in 2020

WHO „Global progress report on HIV, viral hepatitis and sexually transmitted infections, 2021“ (2021)

Epidemiology - Europe

Figure 4a. Rates of confirmed gonorrhoea cases per 100 000 population by year in EU/EEA countries reporting consistently, 2014–2023

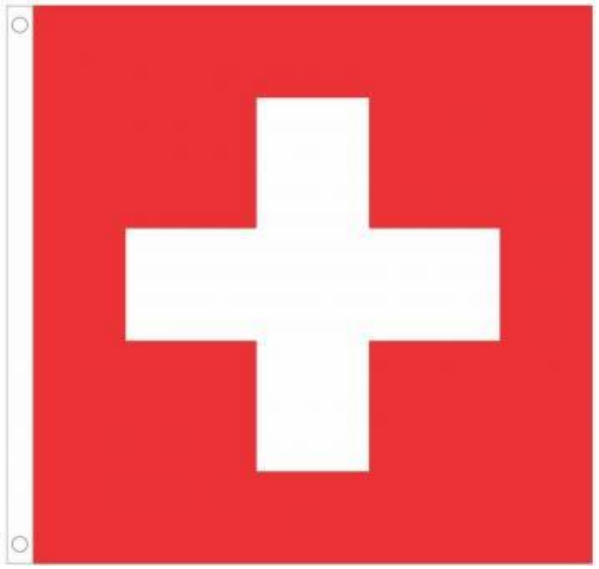


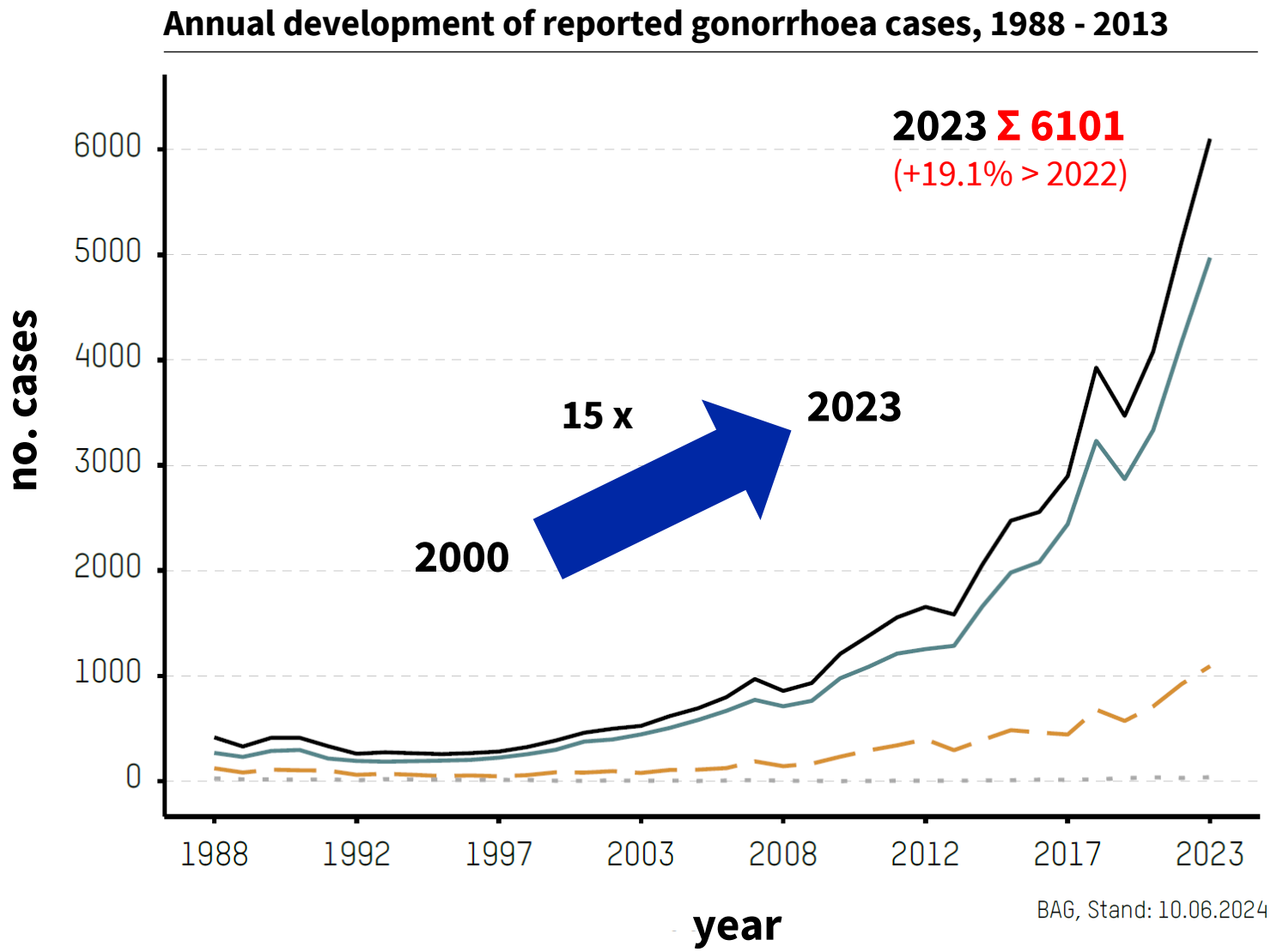
Source: Country reports from Bulgaria, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Malta, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain and Sweden.

Confirmed cases **2020**: 39’170

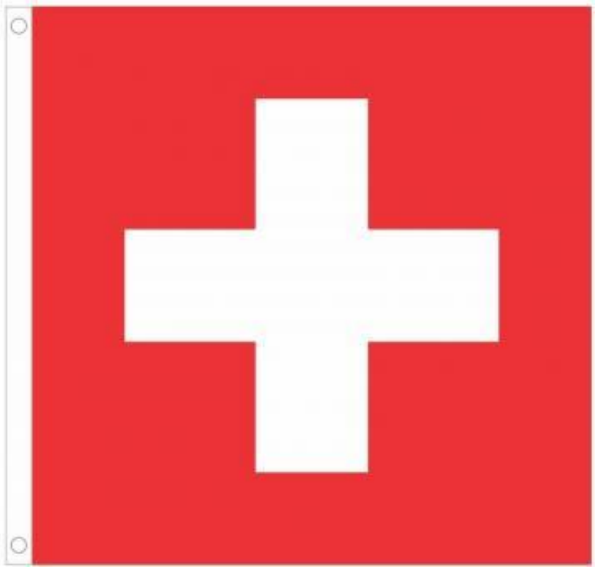
2023: 96’969

Epidemiology - Switzerland





Epidemiology - Switzerland



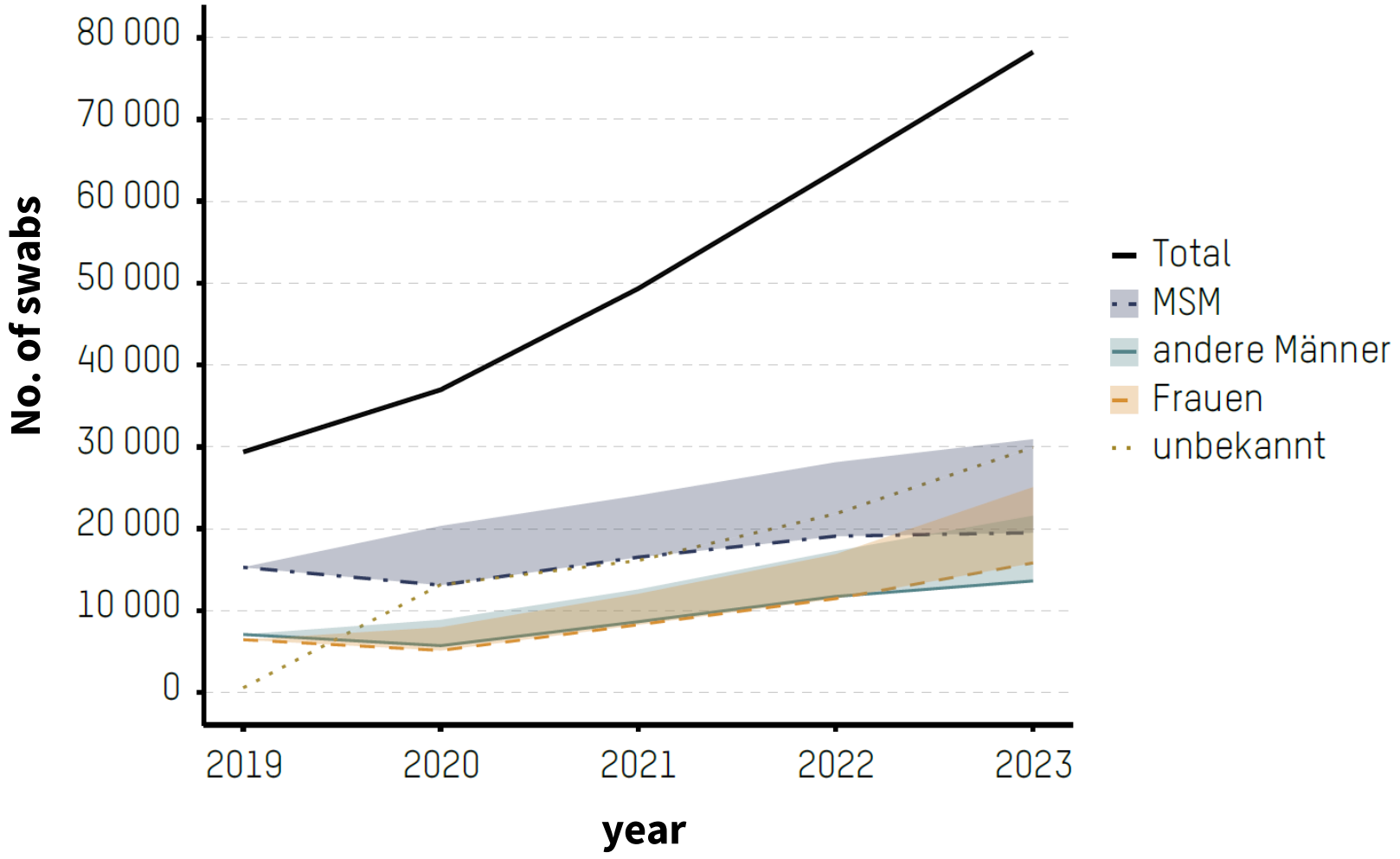
Incidence rates (reported cases/100’000), 2019 - 2023

Grossregion	Diagnosejahr				
	2019	2020	2021	2022	2023
Genferseeregion	46,9	46,7	67,7	80,6	97,5
Espace Mittelland	27,2	26,8	29,1	37,8	46,8
Nordwestschweiz	42,4	32,4	38,2	45,1	52,6
Zürich	94,4	82,1	84,9	105,7	124,1
Ostschweiz	26,5	19,1	21,5	30,4	33,6
Zentralschweiz	31,6	27,2	28,1	35,9	45,2
Tessin	31,3	17,7	27,0	36,4	42,9
Liechtenstein	15,5	10,2	12,7	25,2	25,2
Ganze Schweiz und Liechtenstein	**45,4**	**39,8**	**46,5**	**57,8**	**68,9**

BAG, Stand: 10.06.2024

Epidemiology - Switzerland

No. of swabs taken for N.g. and/or C.t. testing in CH, 2019 - 2023

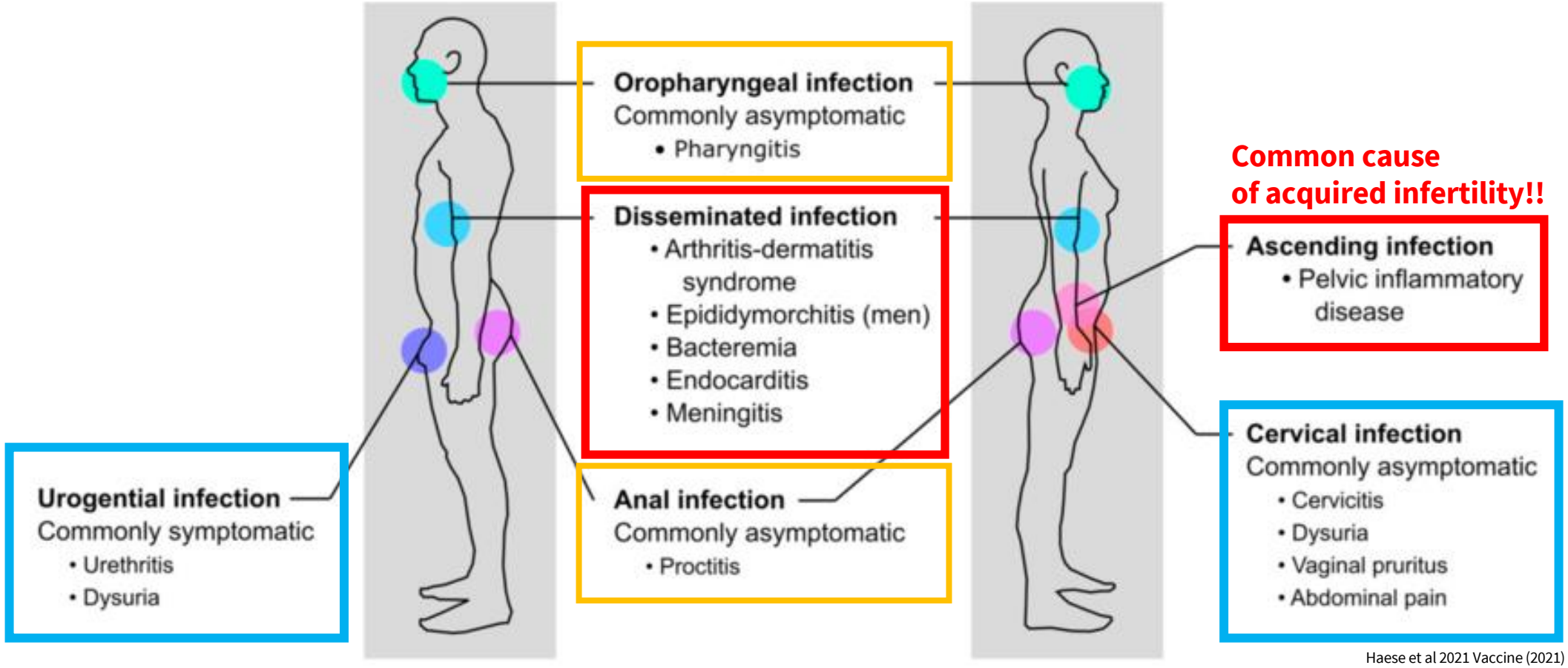


BAG, Stand: 10.06.2024

Gonorrhoea - clinical manifestations

→ Galen (Greek physician, 131 - 200 AD): “gonorrhea”, “unwanted discharge of semen”

→ Affects mucous membranes of urethra, cervix , less frequently rectum, oropharynx, conjunctiva



Haese et al 2021 Vaccine (2021)

Gonorrhoea - clinical manifestations

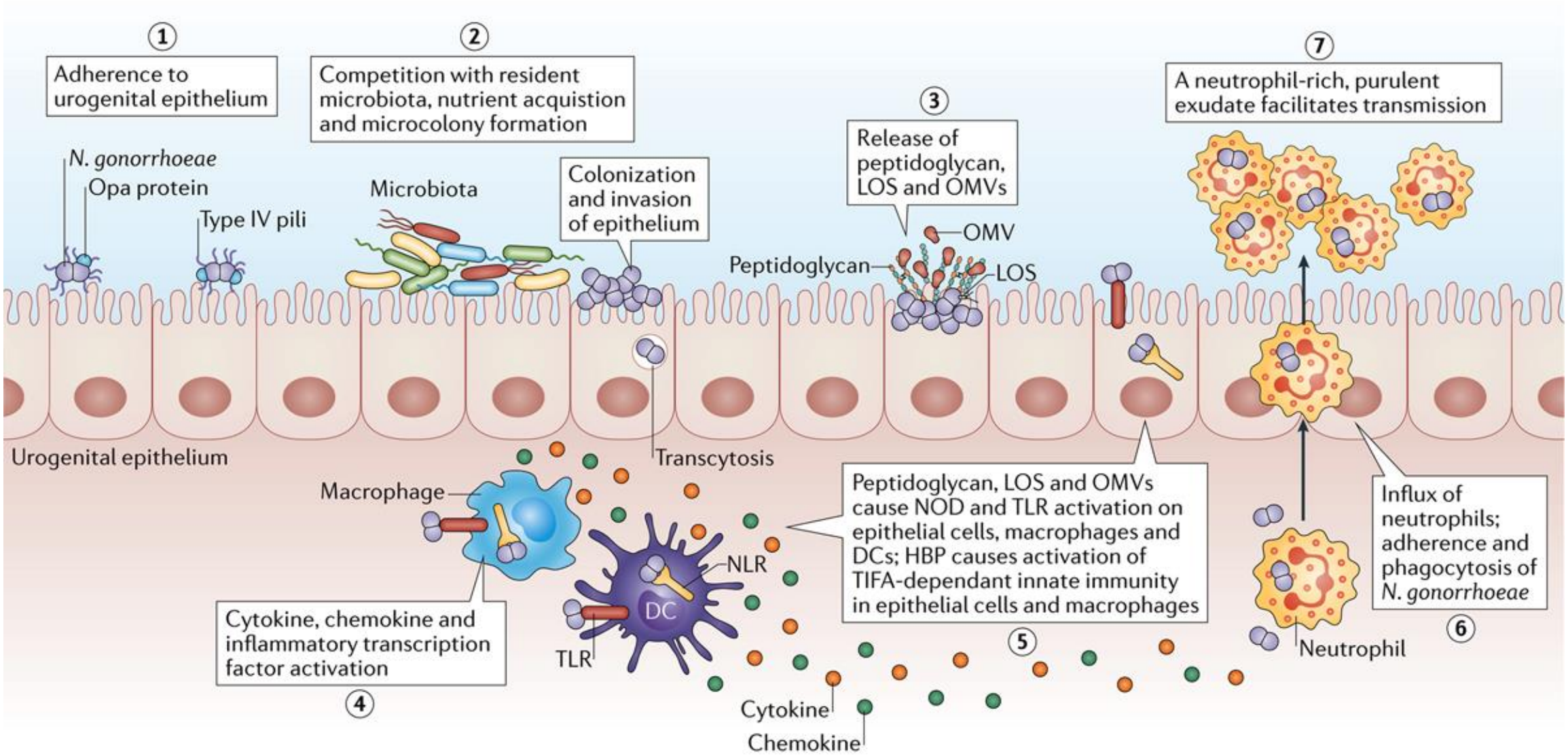
→ **Neonatal conjunctivitis**

→ Historically, common cause of blindness

→ Credé prophylaxis (1% $AgNO_3$)

→ prenatal screenig, antimicrobial treatment

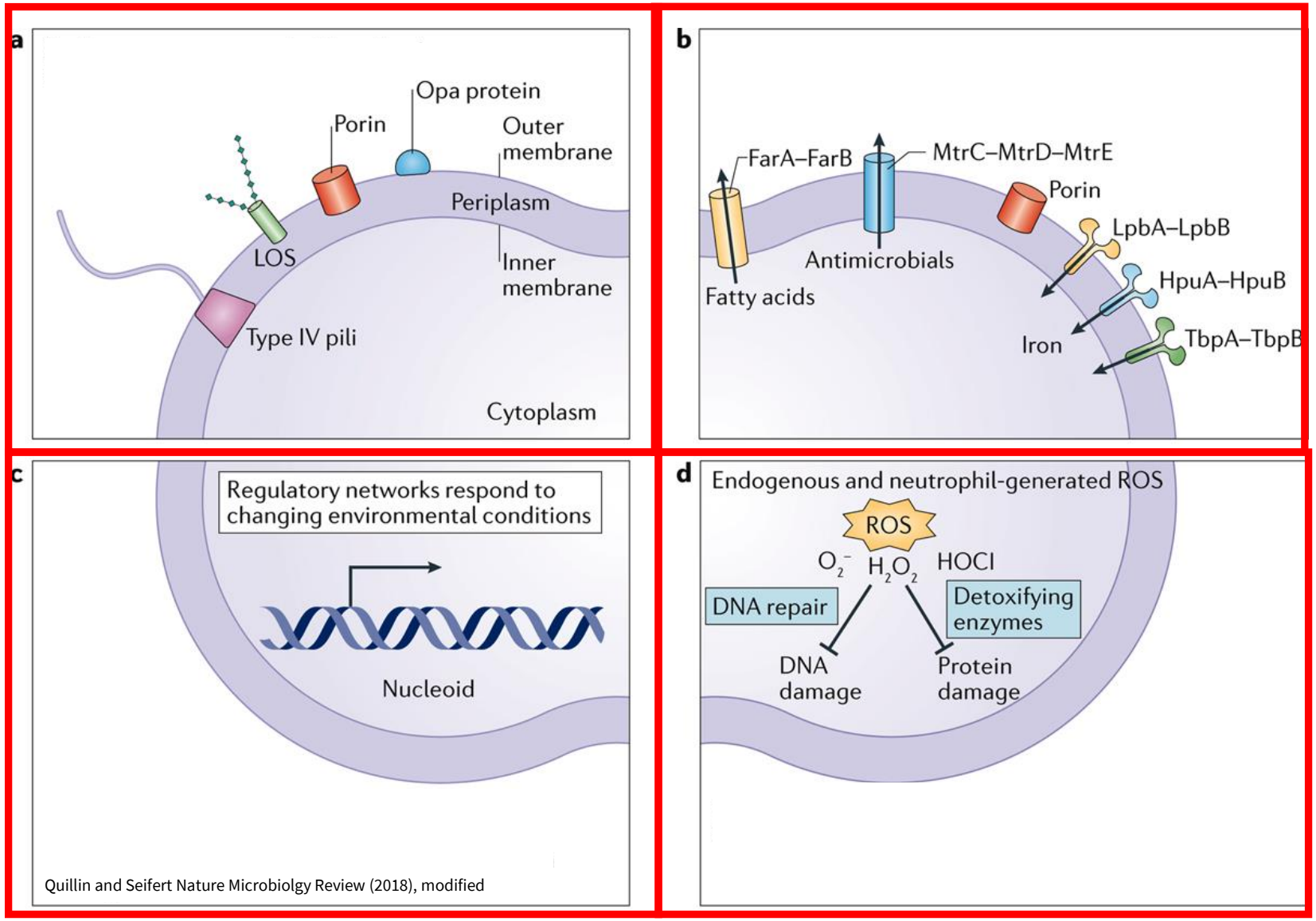
N. gonorrhoeae – infection



Quillin and Seiffert Nature Microbiolgy Review (2018)

Gonorrhoea - pathogenicity factors

Adhesion, invasion, immun evasion



Quillin and Seifert Nature Microbiolgy Review (2018), modified

Transcriptional Response to changing environmental conditions

Nutritional Limitations Efflux/influx

Enzymatic protection

N. gonorrhoeae diagnostics @IMM

Patient sample

Swabs
(urethral, pharyngeal, rectal, cervical)
Urine
…

Molecular Diagnostics

C.t/N.g. (COBAS)

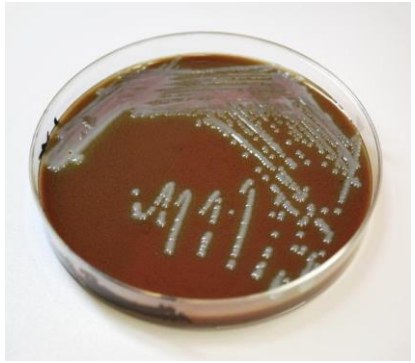
***in house* STD Multiplex PCR panel**
N. gonorrhoeae
C. trachomatis/*C.t.* LGV
M. genitalium +
M.g. macrolide resistance
T. pallidum
H. ducreyi

GeneXpert

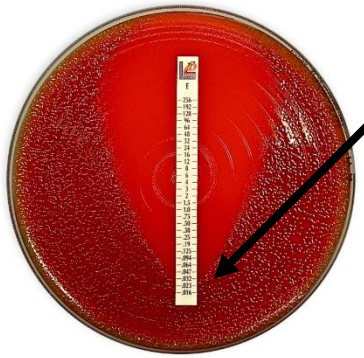
N. gonorrhoeae
C. trachomatis

Bacteriology

Culture



phenotypic antimicrobial susceptibilty testing



minimum inhibitory concentration (MIC)

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

resistant? **susceptible?**

Treatment recommendations

(historically: mercury, urethral lavage w/ hot water, indonesian pepper, tree extracts, heat treatment in «fever cabinets»

Guidelines Swiss Society of Infectious Diseases:

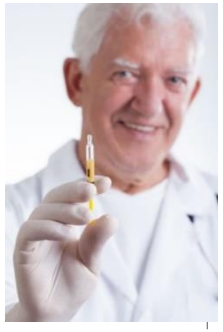
→ **Uncomplicated urogenital, anorectal or pharyngeal gonorrhoea:** **Ceftriaxone (1st line)**

→ **Microbiological control 7d after treatment => culture and resistance testing if symptoms persist**

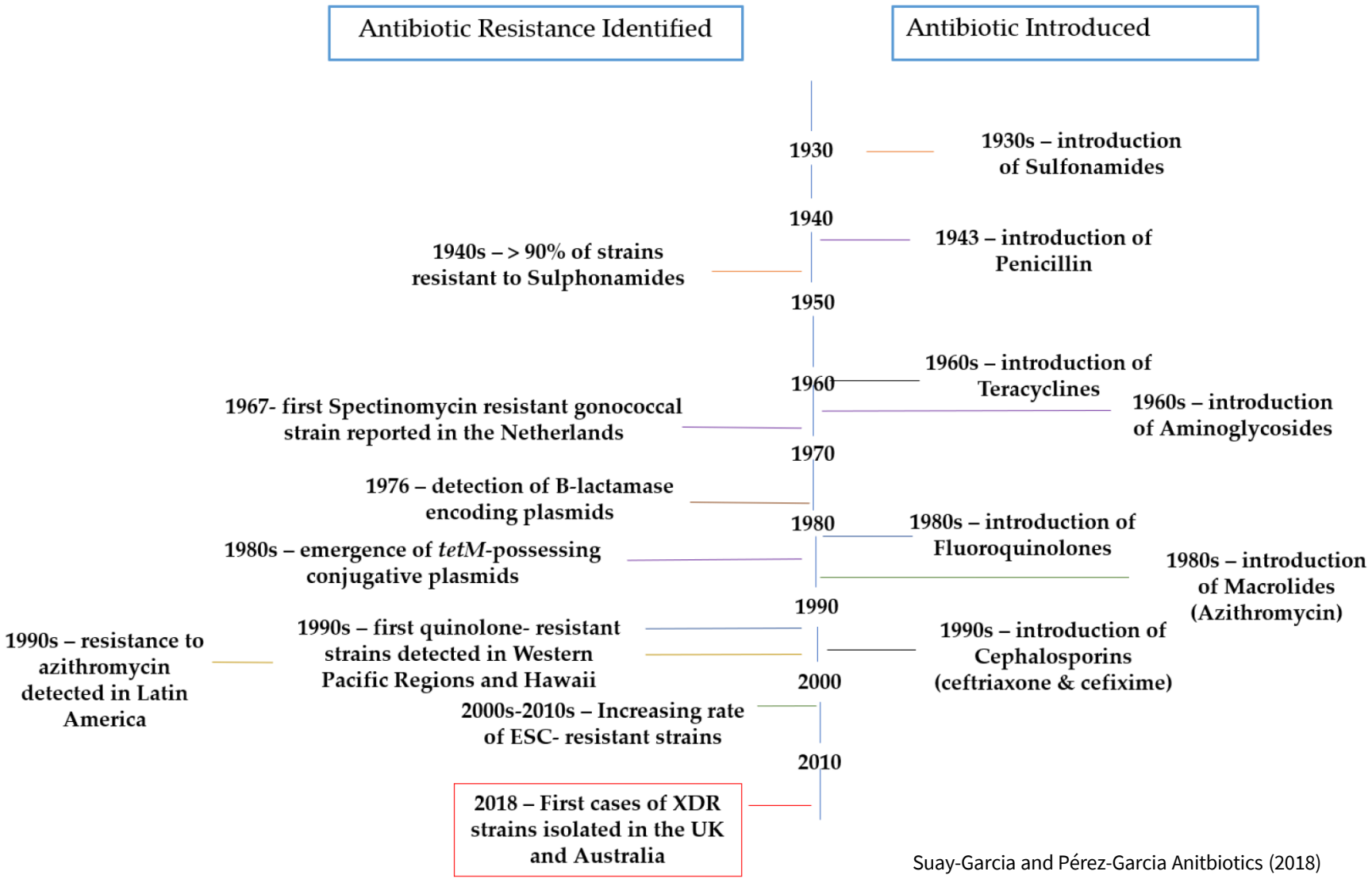
→ **Allergy to cephalosporins:** **gentamicin + azithromycin**

cefixime + azithromycin **(2nd line)**

ciprofloxacin



Evolution of antimicrobial resistance in *N. gonorrhoeae*



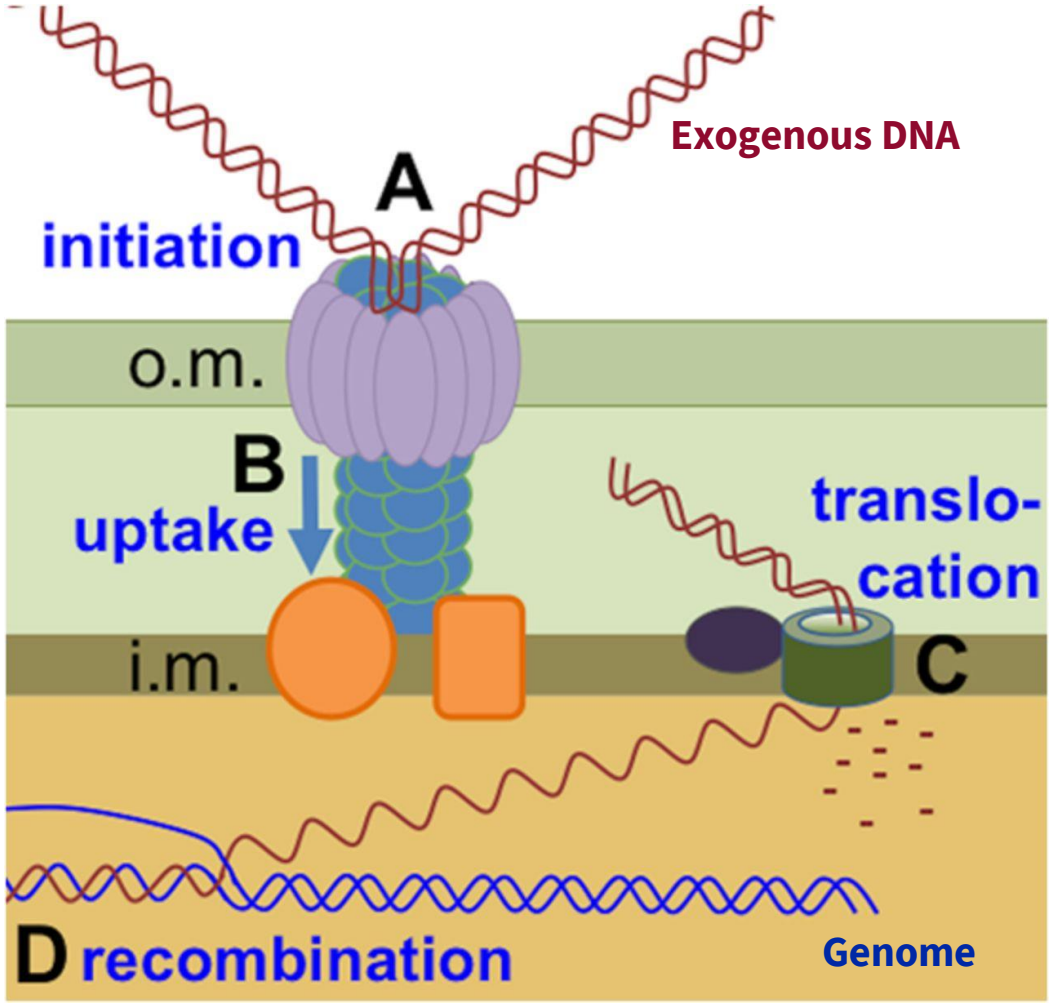
Suay-Garcia and Pérez-Garcia Anitbiotics (2018)

The Guardian

UK man has world-first case of super-strength gonorrhoea

Public Health England say case is first global report of strand resilient to main antibiotic care

Acquisition of drug resistance through transformation



→ **Transformation:** the ability to directly uptake exgenous (chromosomal) DNA and to integrate it into the genome («recombination»)

genetic alterations, accumulation of mutations, **resistance**

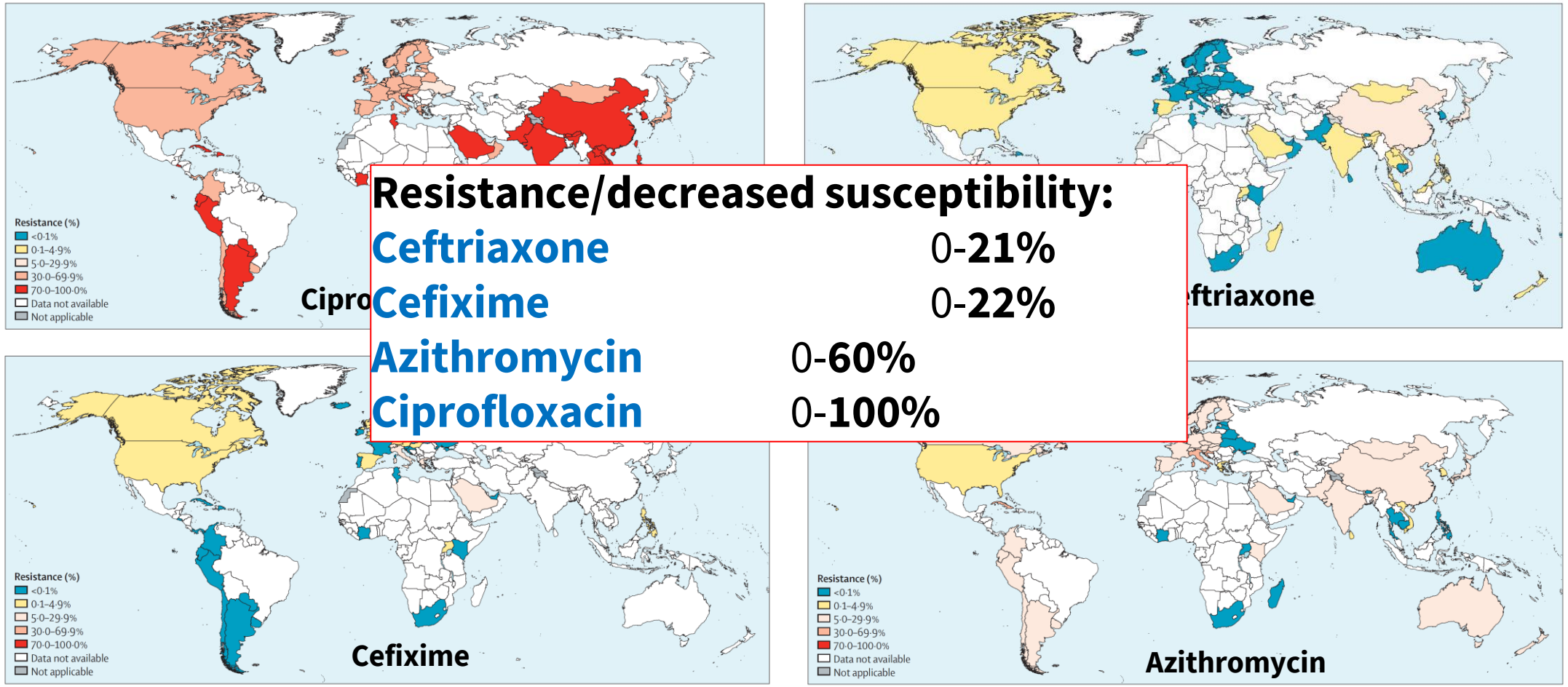
N. gonorrhoeae antimicrobial resistance on a global scale

WHO global antimicrobial resistance surveillance for *Neisseria gonorrhoeae* 2017–18: a retrospective observational study

Magnus Unemo, Monica M Lahra, Martina Escher, Sergey Eremin, Michelle J Cole, Patricia Galarza, Francis Ndowa, Irene Martin, Jo-Anne R Dillon, Marcelo Galas, Pilar Ramon-Pardo, Hillard Weinstock, Teodora Wi

***Lancet Microbe* 2021; 2: e627–36**

N. gonorrhoeae antimicrobial resistance on a global scale

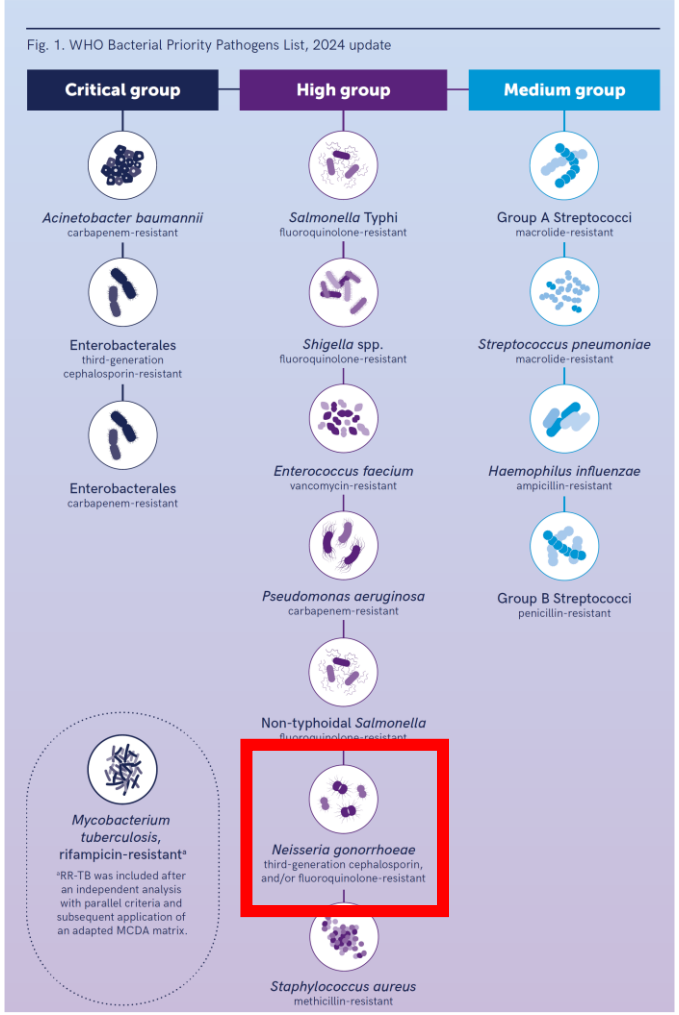


Resistance/decreased susceptibility:

Ceftriaxone	0-**21%**
Cefixime	0-**22%**
Azithromycin	0-**60%**
Ciprofloxacin	0-**100%**

Unemo *et al* Lancet Microbe (2021) mod.

N. gonorrhoeae – a WHO high priority pathogen



"The inclusion of these pathogens in the list **underscores their global impact in terms of burden, as well as issues related to transmissibility, treatability, and prevention options**. It also reflects the R&D pipeline of new treatments and emerging resistance trends."

"**The WHO BPPL acts as a guide for prioritizing R&D and investments in AMR, emphasizing the need for regionally tailored strategies to effectively combat resistance**. It targets developers of antibacterial medicines, academic and public research institutions, research funders, and public–private partnerships investing in AMR R&D, as well as policy-makers responsible for developing and implementing AMR policies and programs."

https://www.who.int/publications/i/item/9789240093461

N. gonorrhoeae – a local study on antimicrobial resistance (Jan 2019 – Dec 2021)

Original Research Article

INTERNATIONAL JOURNAL OF STD & AIDS

Phenotypic and genotypic characterization of *Neisseria gonorrhoeae* isolates among individuals at high risk for sexually transmitted diseases in Zurich, Switzerland

Christian Jünger[1], Frank Imkamp[2], Suraj Balakrishna[1], Marina Gysin[2], Klara Haldimann[2], Silvio D Brugger[1], Thomas C Scheier[1], Benjamin Hampel[3], Sven N Hobbie[2], Huldrych F Günthard[1] and Dominique L Braun[1]

International Journal of STD & AIDS
2024, Vol. 0(0) 1–9

DOI: 10.1177/09564624241230266
journals.sagepub.com/home/std
Sage

Baseline characteristics

	All: *N* = 142	Culture positive: *N* = 23
Number of positive *N. gonorrhoeae* tests	182	23
Sex (female; Male)	1; 141	0; 23
Sexual orientation MSM	141 (99.5%)	23 (100%)
Median age	41 (25-63)	43 (26-56)
Symptoms attributable to STD (yes)	46 (35.1%)	10 (45.5%)
Symptoms (N/A)	11	1
Exposition in Switzerland	68 (87.2%)	12 (92.3%)
Exposition (N/A)	64	10
Clinical cure	134 (100%)	20 (100%)
Clinical cure (N/A)	8	3
Microbiological cure	122 (100%)	16 (100%)
Microbiological cure (N/A)	20	7
HIV (positive)	118 (83.7%)	17 (73.9%)
HIV (N/A)	1	0

Swiss HIV Cohort Study (SHCS)
Zurich Primary HIV Infection Cohort Study (ZPHI)
Swiss PrEPared study

PCR/culture: STI screening every 3-6 month or symptoms

Treatment: Ceftriaxone (N=79), Cef+Genta (N=2), Cef + Azi (N=61)

The study workflow

142 individuals/
182 N.g. PCR positive
samples

Phenotypic analysis

Determination
Minimum Inhibitory Concentration
(MIC) by E-Test and agar dilution
(12 drugs)

Resistant?
Susceptible?

MATCH??

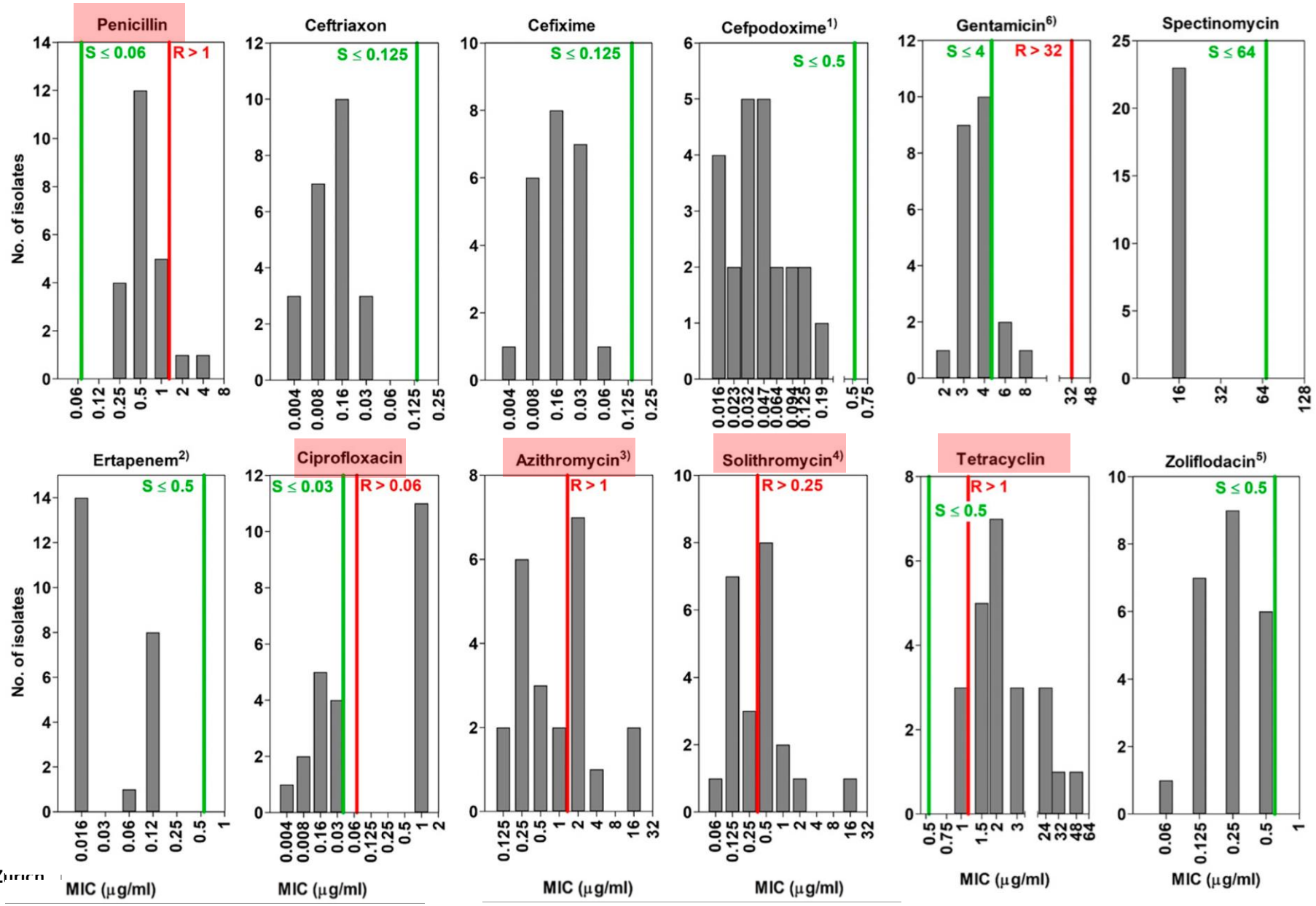
Genotypic analysis

Illumina Sequencing

Sequence analysis

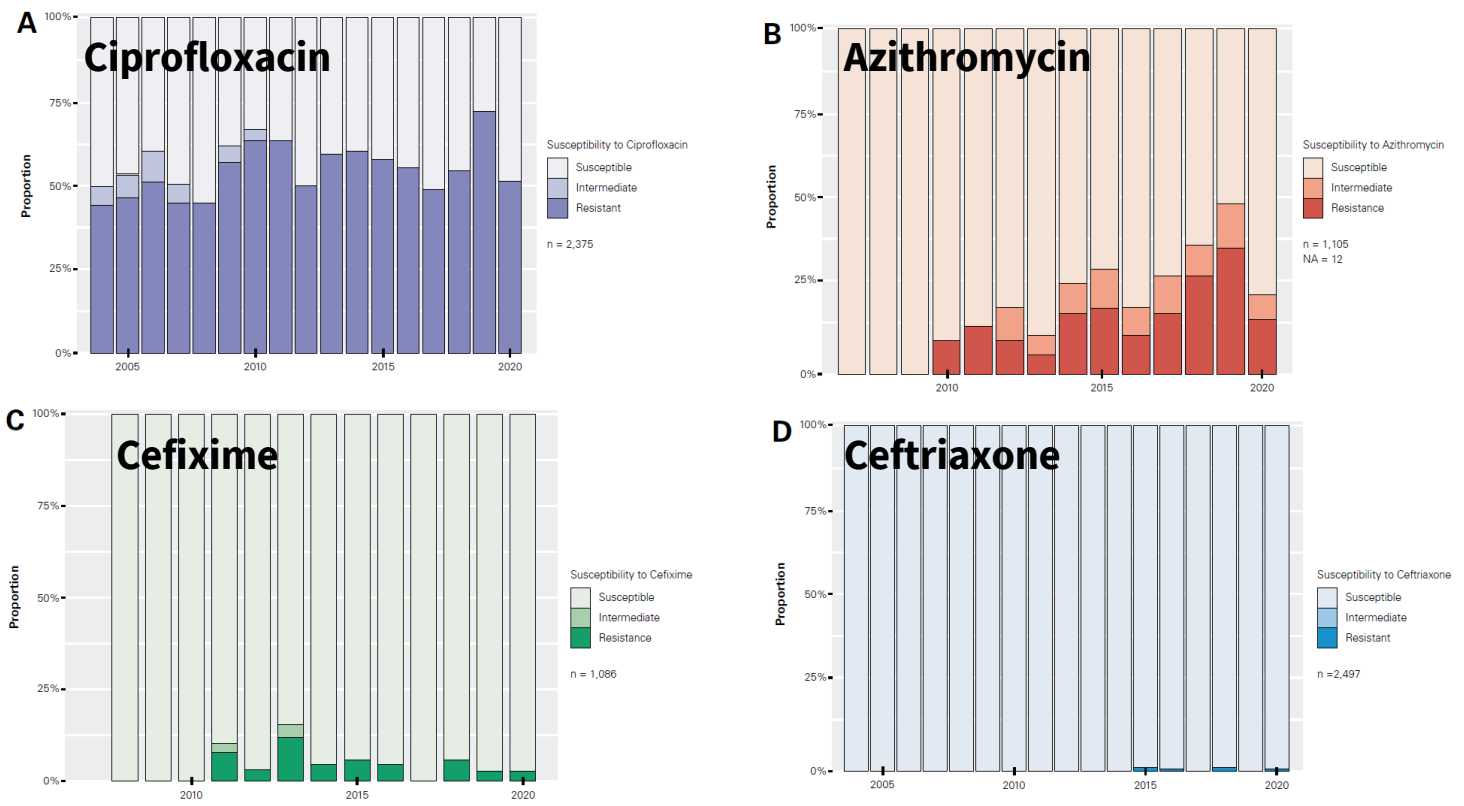
Identification of resistance
determinats and mutations
(CARD database)

MIC distributions of tested antibiotics



N. gonorrhoeae resistance in Switzerland

Figure 1: *Neisseria gonorrhoeae* isolates in Switzerland, 2004–2020, with results of antimicrobial susceptibility testing. Panel A, ciprofloxacin; B, azithromycin; C, cefixime; D, ceftriaxone.



Federal Office of Public Health and Federal Food Safety and Veterinary Office. Swiss Antibiotic Resistance Report 2022.

Genotypic characterisation – identified resistance determinants (WGS)

Penicillin:

- Non-WT, non-mosaic PBP-2 (*penA*)
- PorA (L421P)
- PorB (G120K, A121N/D)
- MtrR repressor (A39T, G45D)
- *mtrR* promoter
- TEM-1 β-lactamase

Azithromycin:

- 23S rRNA gene (C2611T, A2059G)
- *ermF* (Methylase)
- Mosaic *mtrD* promoter
- Mosaic MtrD
- *rplD* (G70D)

Ciprofloxacin:

- ParC (D86N, S87R, E91G)
- GyrA (S91F, D95A/G)

Tetracyclin:

- RpsJ (V75M)
- TetM

→ **>98.9% match Genotype/Phenotype**

→ **3 isolates with TEM-1 BL did not display expected penicillin resistance**

S

Study summary

→ **low number of analysed isolates**, local epidemiology of a high-risk setting

→ **No resistance against 1st line treatment (Ceftriaxone)**

→ **44% resistance rate for azithromycin;** 2nd line treatment! (SSI guidelines)

→ **Alternative treatment options** (e.g. gentamicin, cefpodoxime, ertapenem, zoliflodacin)

→ **WGS data** are a relieable source **to deduce phenotype of clinical isolates**

N. gonorrhoeae diagnostics – a brief outlook

→ Antimicrobial susceptibilty testing (AST) of isolate is crucial for monitoring resistance evolution

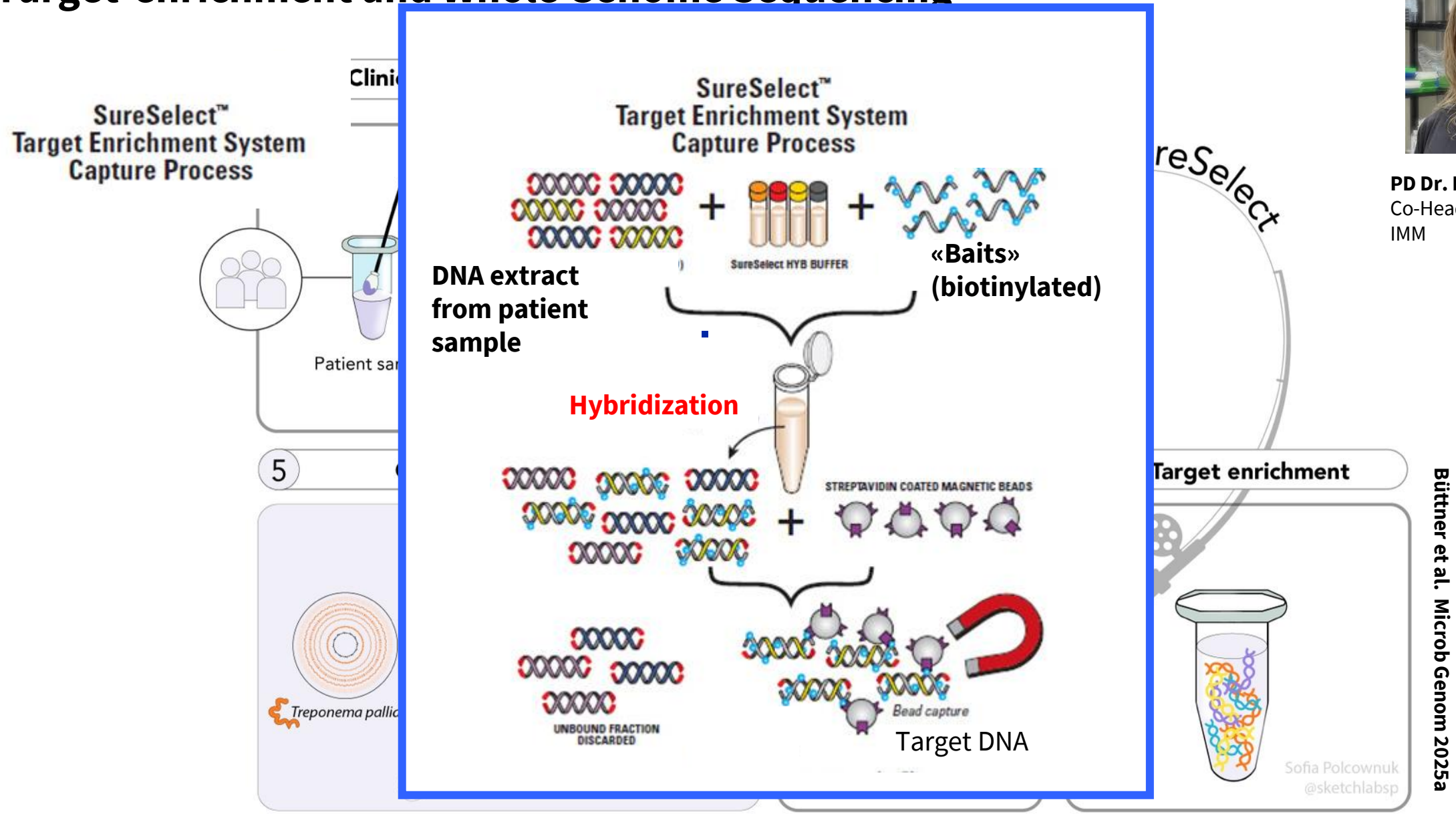
→ Only 1 of 10 confirmed *N. gonorrohoeae* infections is accompanied by AST (source: anreiss.ch)

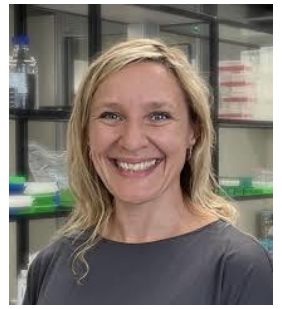
→ **Shift towards Molecular Diagnostics (MD)** for pathogen and resistance detection from patient sample

Cave: non-pathogenic, commensal *Neisseria sp* (extragital infections), multitude of factors determine one phenotype, «gaps» in resistance databases

→ **Metagenomics** from patient samples to determine resistance: feasible; low bacterial load, human DNA background!

Target-enrichment and Whole Genome Sequencing

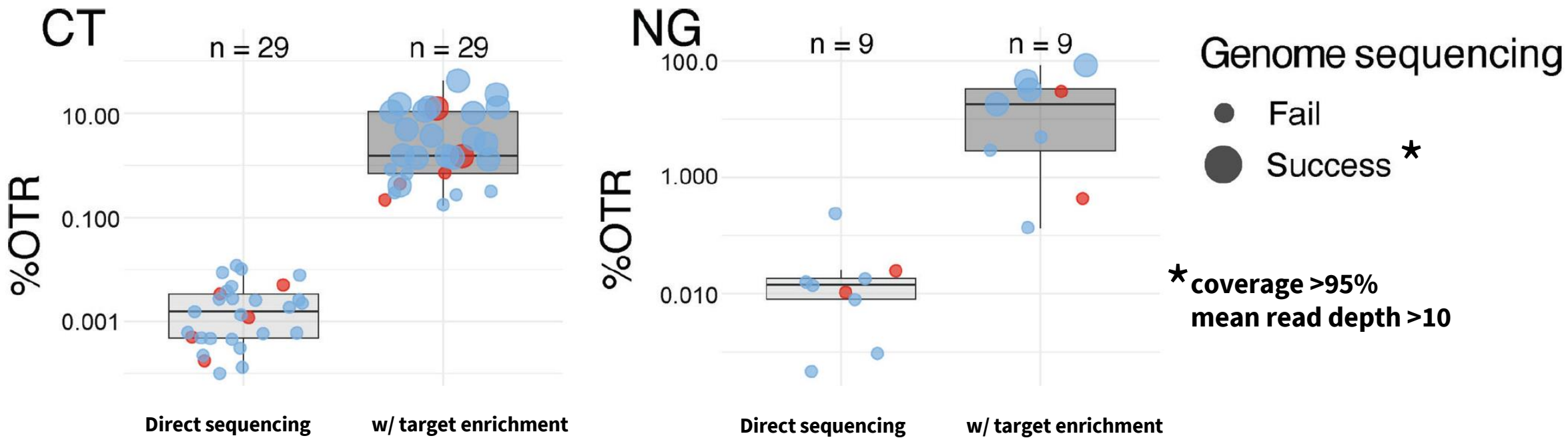




PD Dr. H. Seth-Smith
Co-Head Microbi. Genomics
IMM

Büttner et al. Microb Genom 2025a

Target-enrichment and Whole Genome Sequencing



OTR, on target reads

Büttner et al. Microb Genom 2025a

Thank you for your attention!