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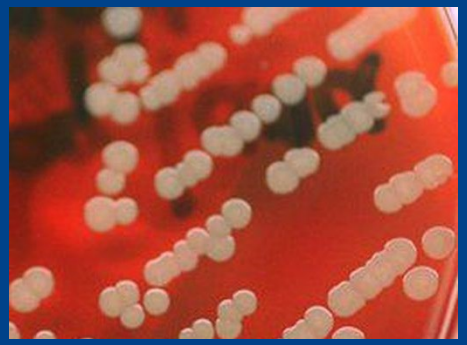
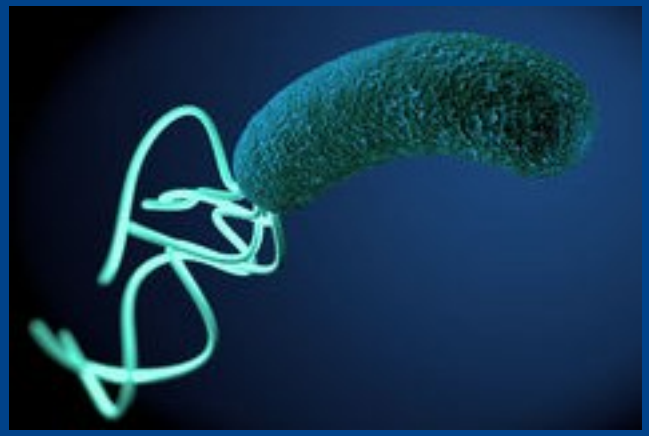
Impact of novel molecular diagnostics on AMR

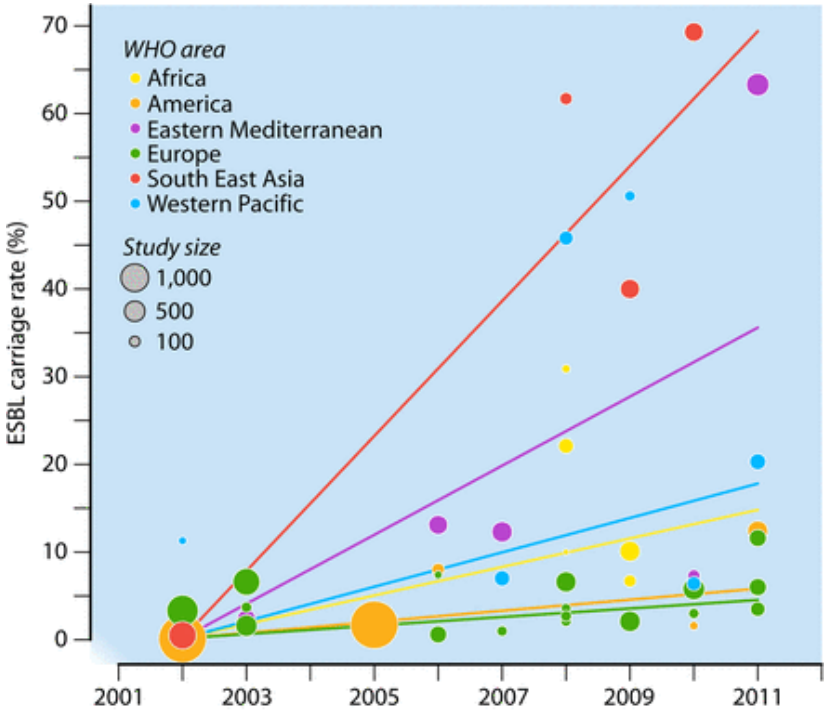
Paul Savelkoul





Dept. medical microbiology

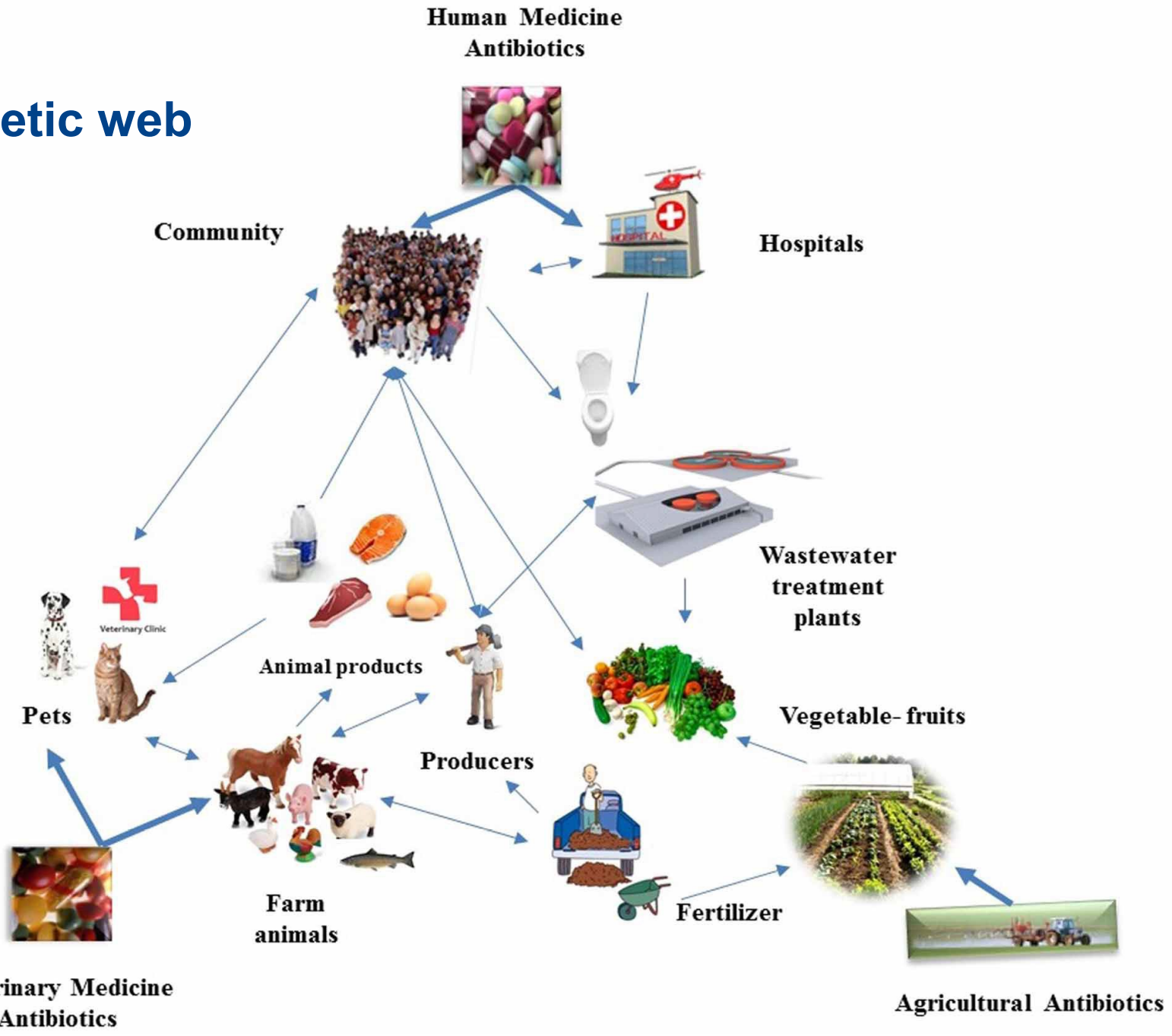
Antimicrobial resistance:
Increasing resistance worldwide



ESBL: Extended Spectrum Beta-Lactamases

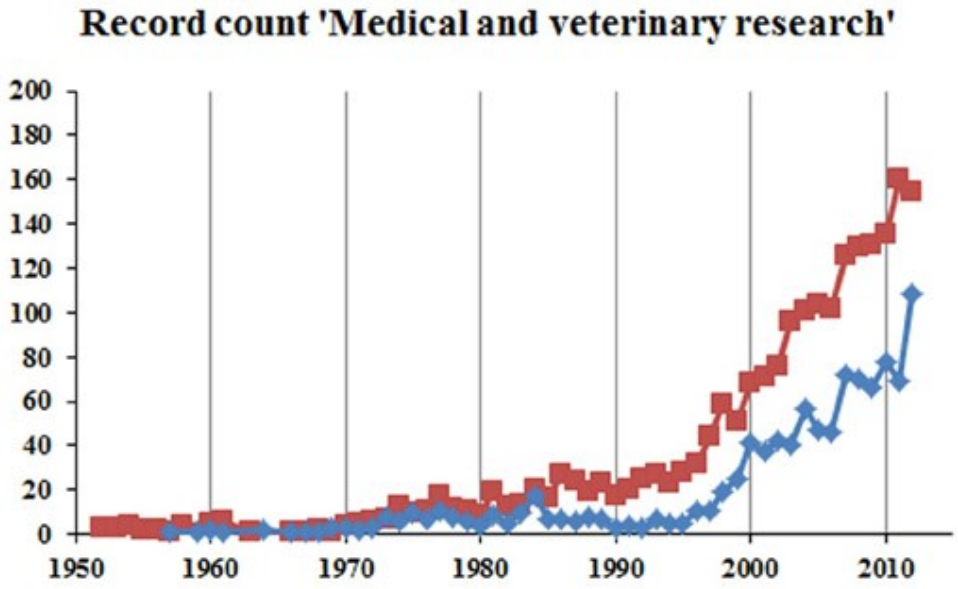
Woerther P.L. Clin Microbiol Rev. 2013;26(4):744-58

The genetic web



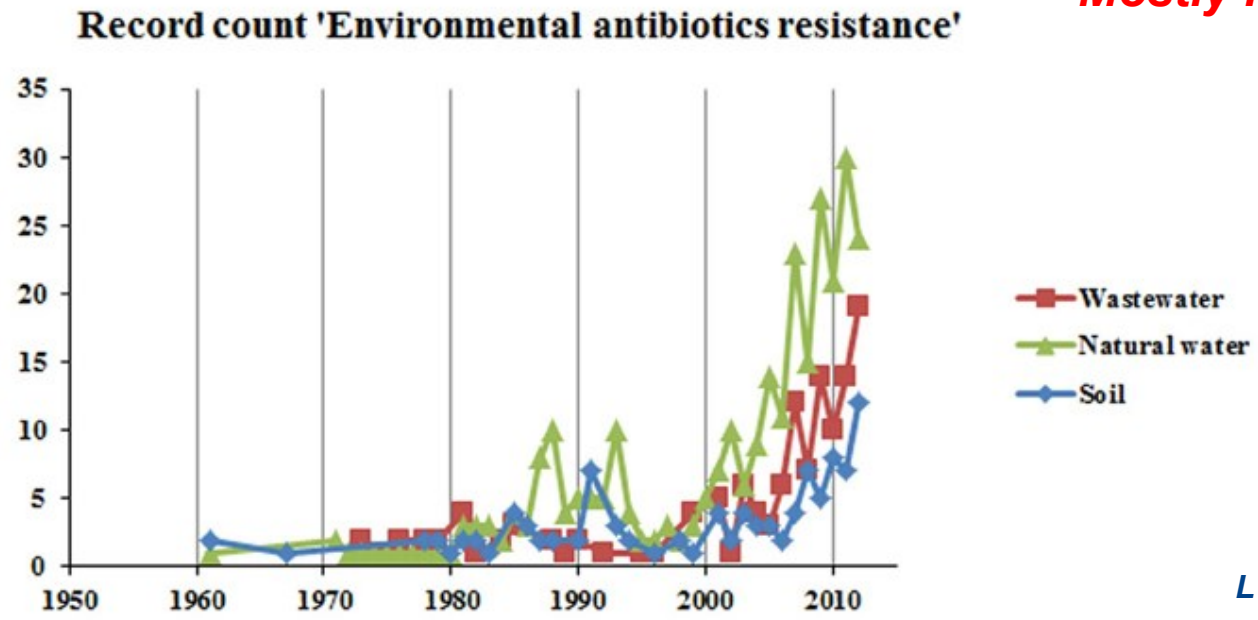
L Cantas, et al, Front. Microbiol, 2013

AMR: worldwide important public health treath



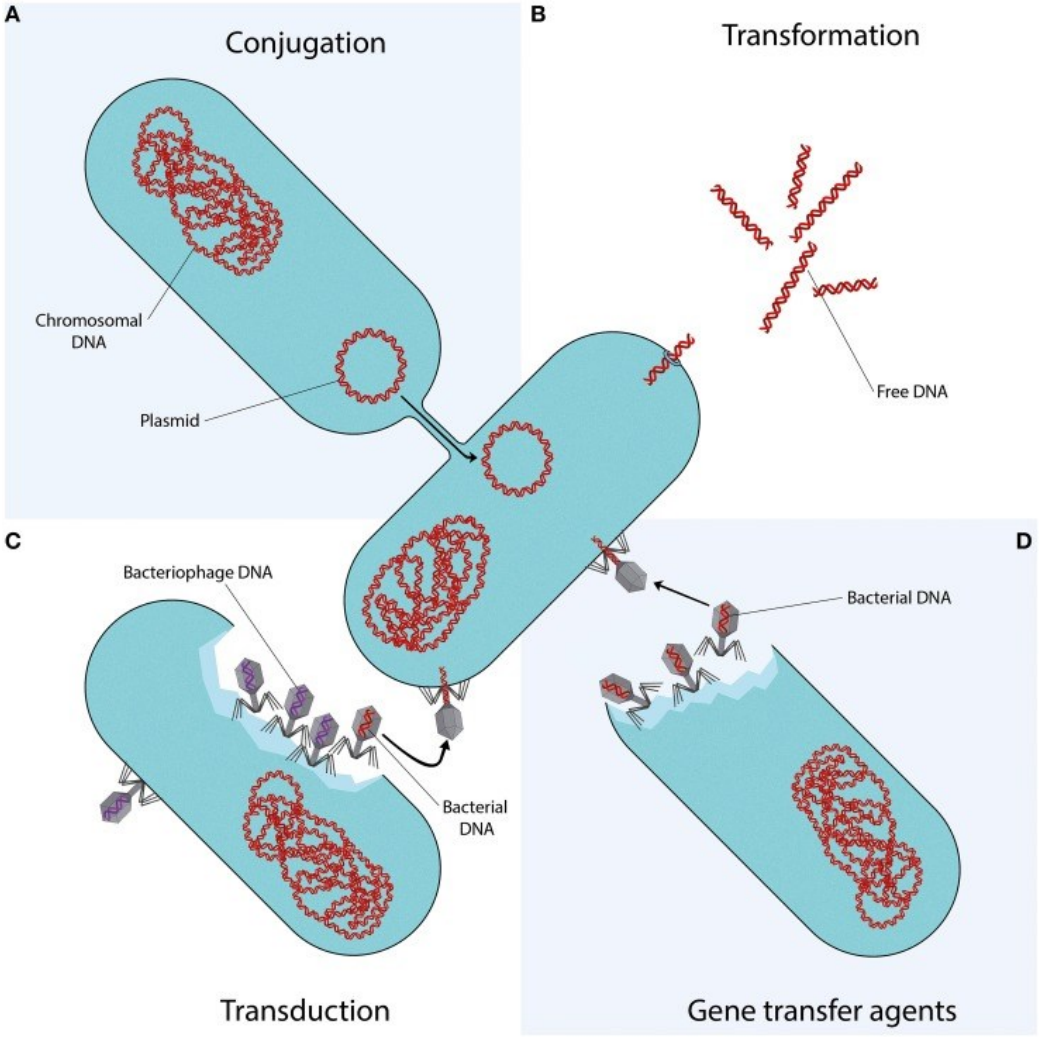
Increase in research papers reflects the increase of the problem of AMR

Mostly focused on clinical isolates



L Cantas, et al, Front. Microbiol, 2013

The genetic content of micro organisms is very dynamic



Antibioticadruk

Wintersdorf et. al. Front Microbiol. 2016

Spread of AMR

Rapid spread of resistant strains

Different routes of transmission

Rapid detection of resistance leads to earlier recognition (infection control)

Rapid detcetion leads to optimal antibiotic treatment

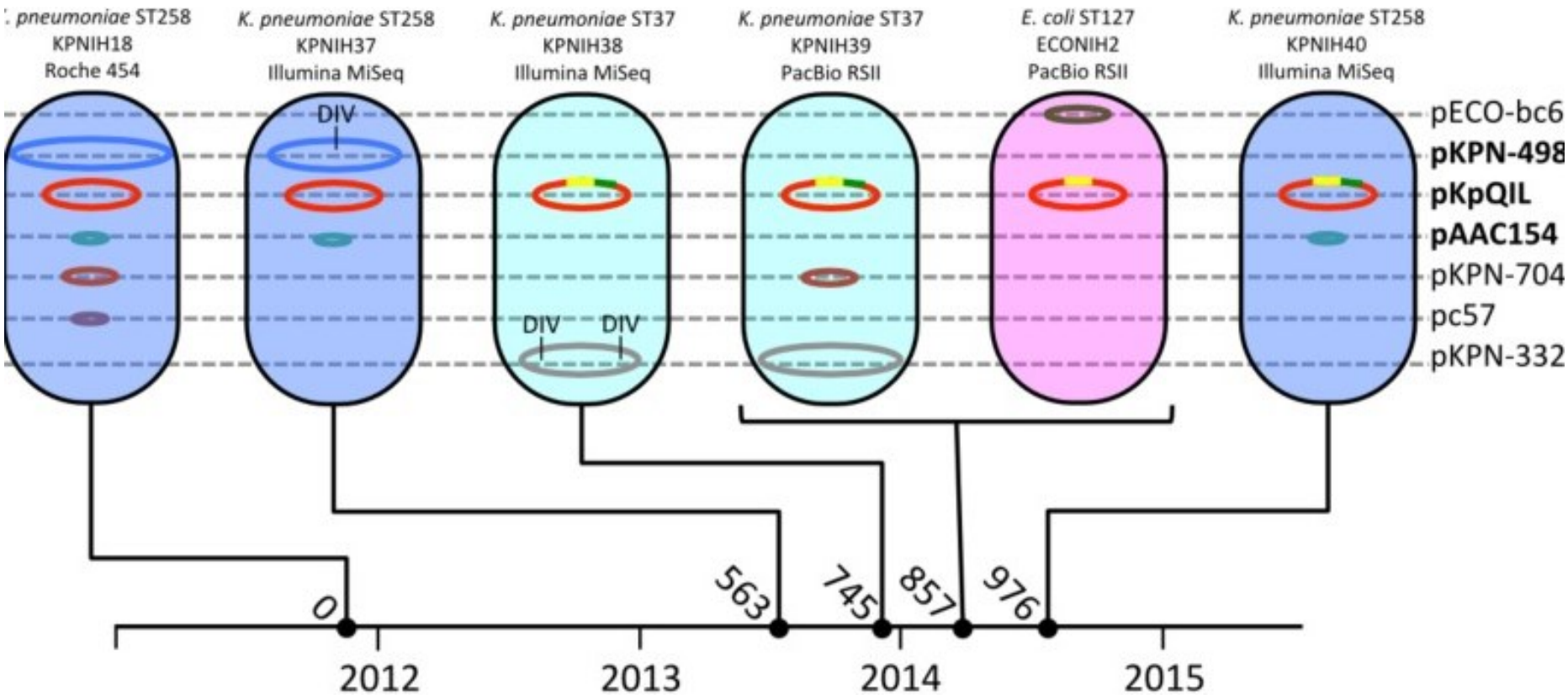
Spread of AMR

Vertical transmission: clonal, specific strain e.g. MRSA

Horizontal transmission: mobile genetic elements, clonal & non-clonal e.g. ESBL

- Large difference in molecular detection
- Different dynamics

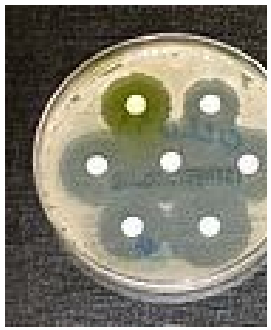
Plasmid Dynamics in KPC-Positive *Klebsiella pneumoniae* during Long-Term Patient Colonization

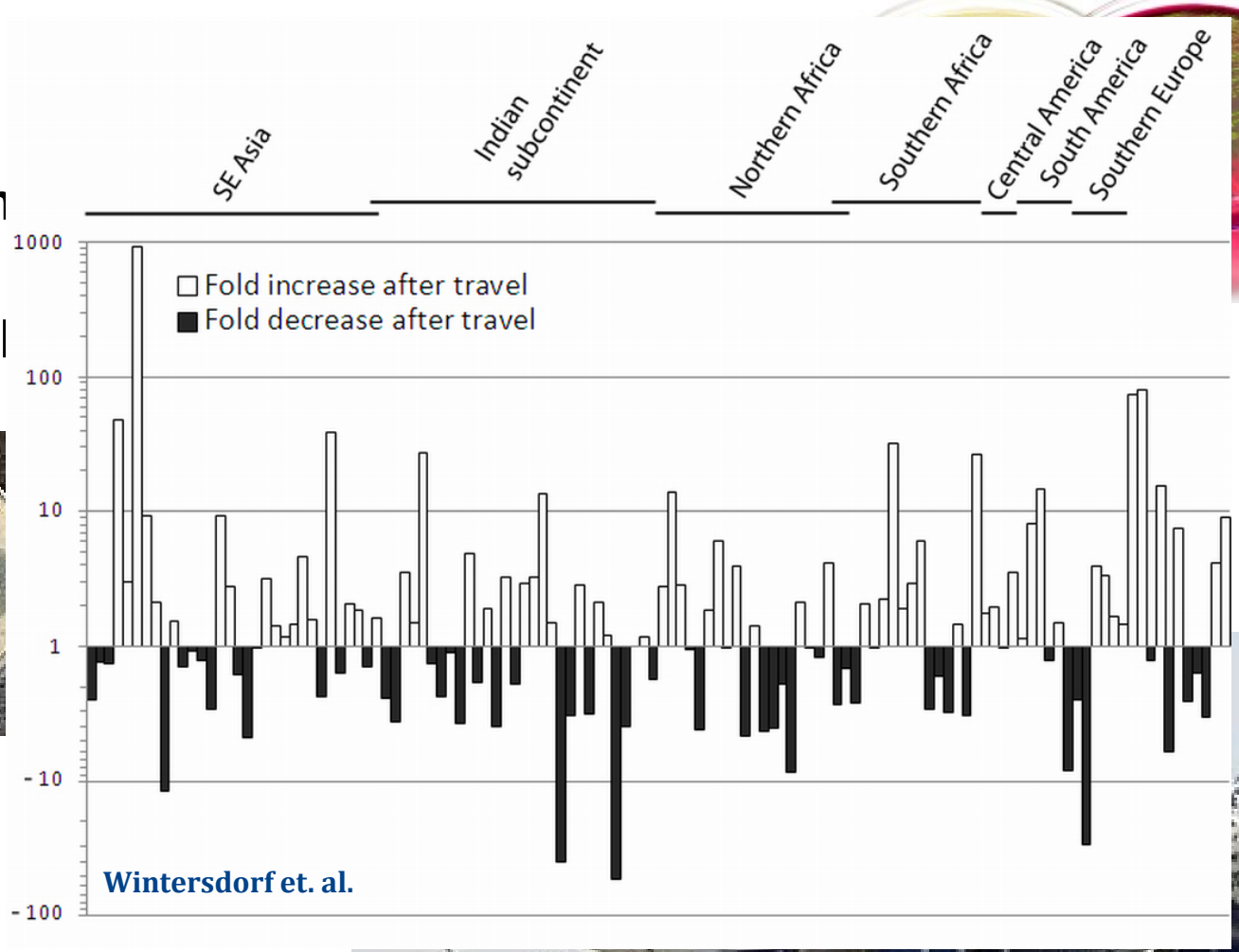


Conlan, S. et. al. 2016 Mbio 7:3, 1-9 e00742-16

Transmission

- Transm
- Recom
- Develo





Advantages of phenotypic sensitivity testing:

- Detection of expression of resistance
- Level of sensitivity (sensitive/intermediate/resistant)
- Detection of unknown resistances

Disadvantages of phenotypic sensitivity testing:

- Takes a long time (24-48h)
- Low level of resistance difficult to detect
- Requires pure culture (can not be used on clinical specimens)
- Some resistance mechanisms are problematic/difficult to identify

Need for Speed

- Adequate treatment

- Reduction of transmission of AMR

Advantages genotypic resistance detection:

- Possible on clinical specimen
- Rapid result
- Suited for bacteria expressing low resistance levels
- Clear results (specific gene)
- Detection of mobile genetic elements

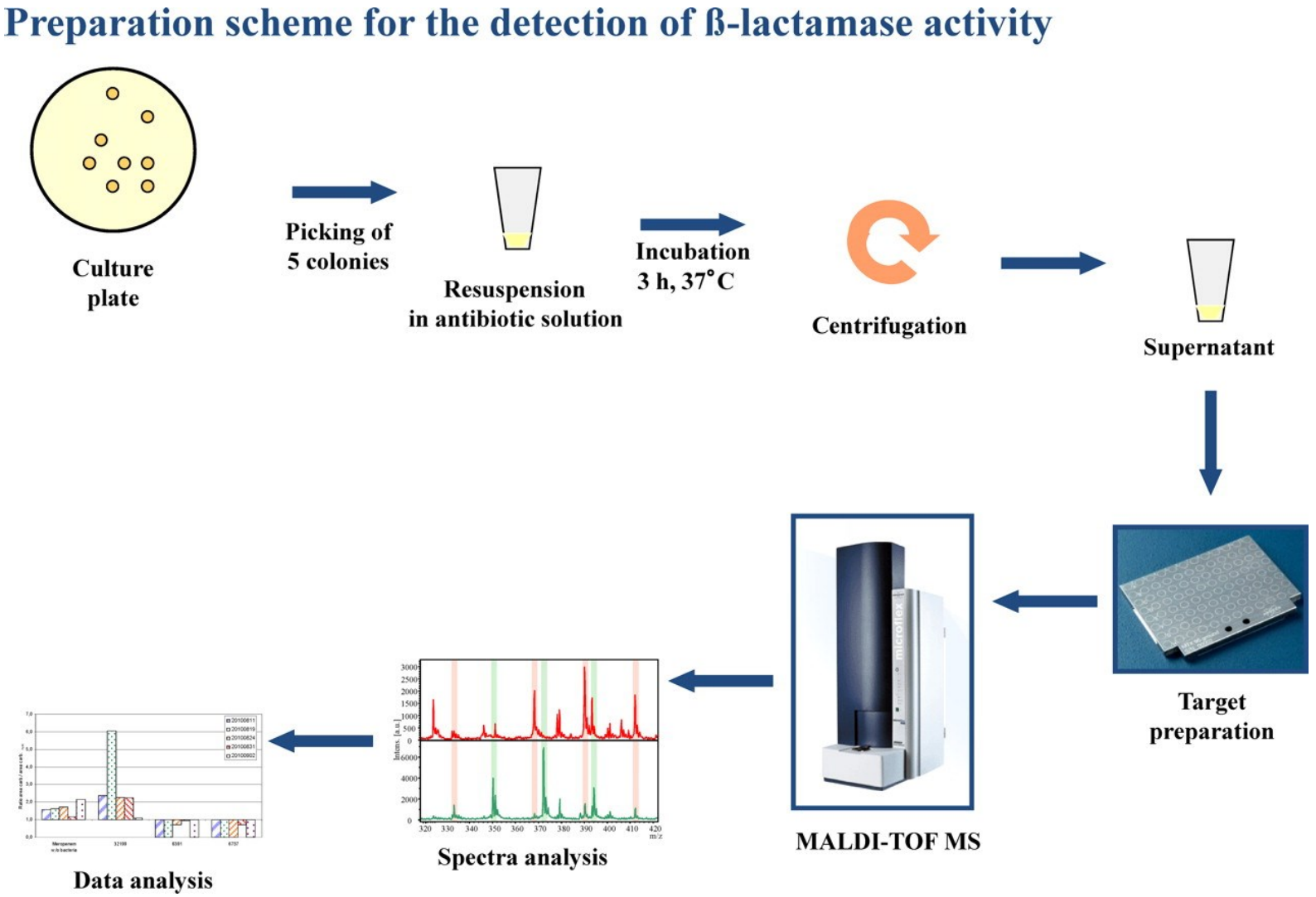
Disadvantages genotypic resistance detection:

- Only known genes will be detected
- Many combinations of PCRs needed with mobile genetic elements
- Not every resistance can be detected
- Resistome probems with clinical specimen
- False negative results due to primer location variants

New molecular developments in detection of resistance

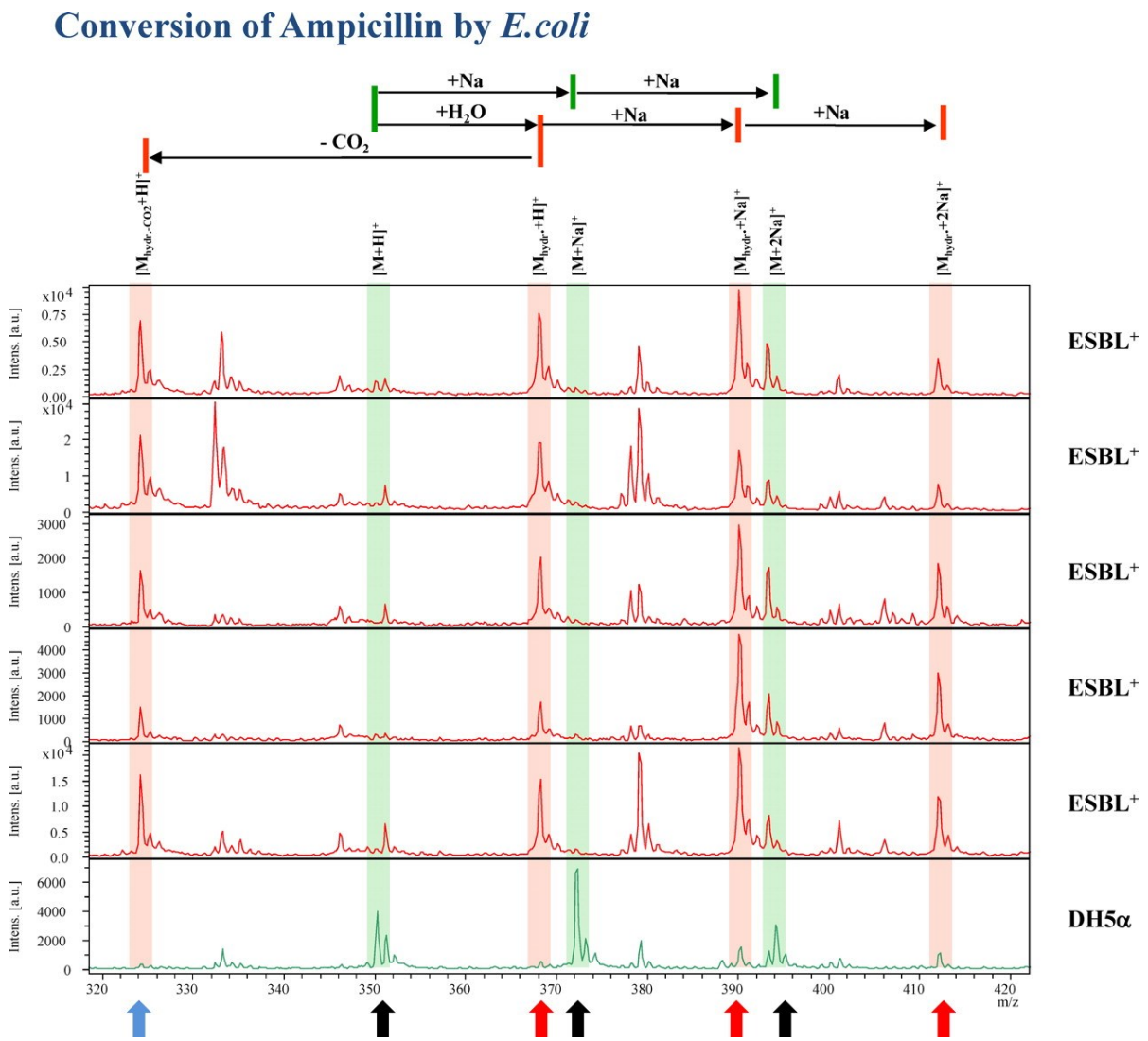
- Malditof
- Extreme PCR
- NGS

Procedure for detection of beta-lactamase metabolites by MALDI-TOF mass spectrometry.



Nathan A. Ledeboer, and Richard L. Hodinka
J. Clin. Microbiol. 2011;49:S20-S24

MALDI-TOF mass spectrogram of ampicillin for five ESBL-producing Escherichia coli strains and one ampicillin-susceptible strain.



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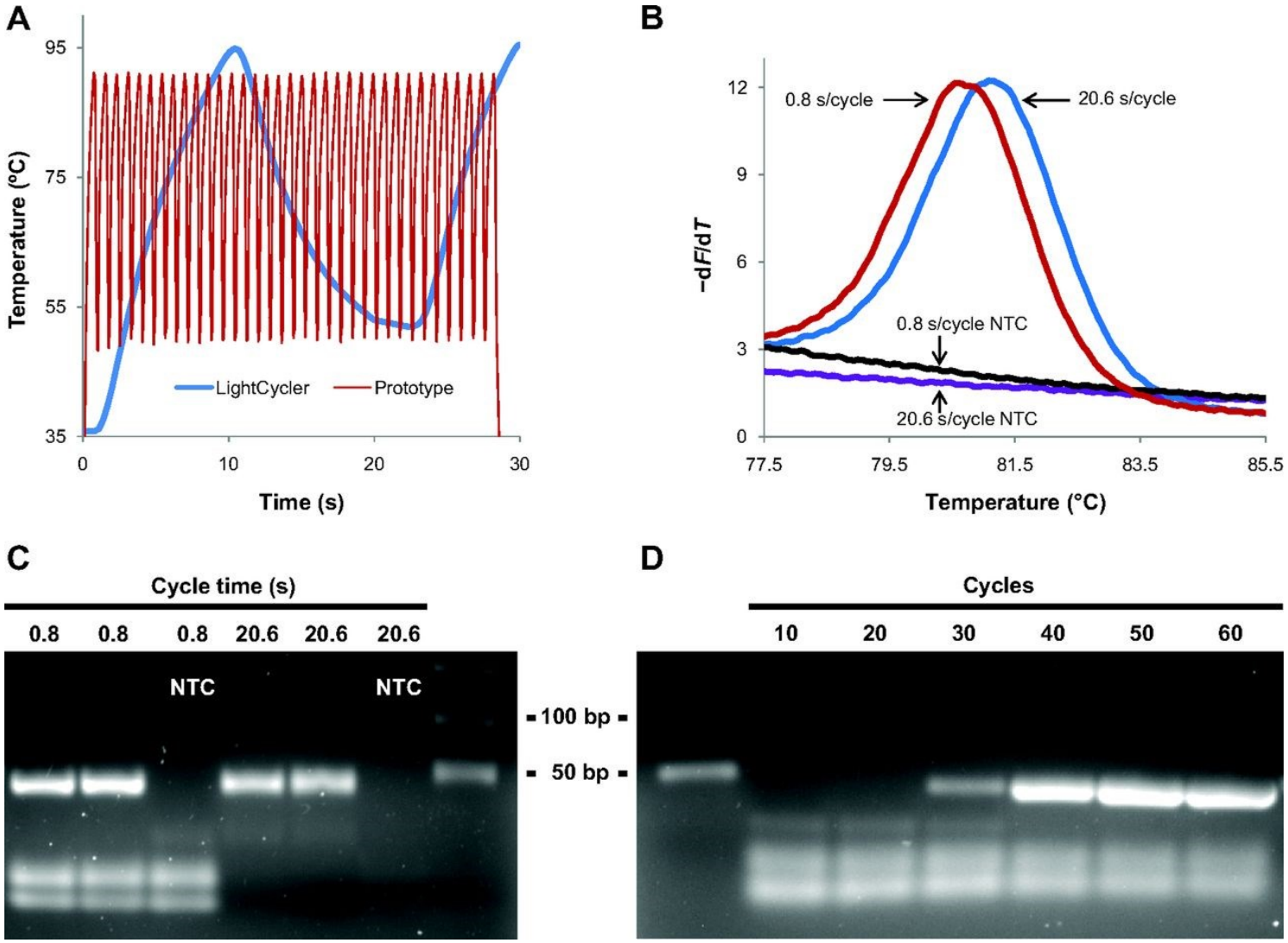
Nathan A. Ledeboer, and Richard L. Hodinka J. Clin. Microbiol. 2011;49:S20-S24

New developments detection of resistance/sensitivity

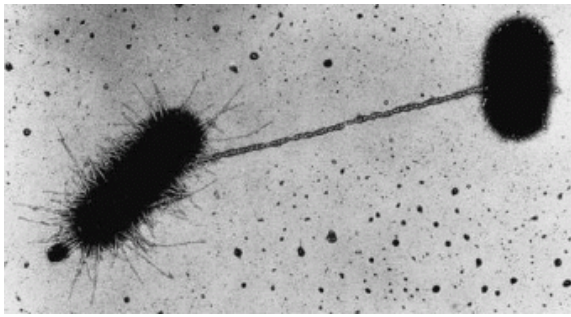


Resistome

- Many micro-organisms may carry resistance genes

- Resistome = “the collection of antibiotic resistance genes in both pathogenic and non-pathogenic bacteria”
- Culturable & non-culturable

  – Microbial communities – potential reservoirs of exchangeable resistance genes



All these ARGs potentially matter
and should be studied

Wright, G.D. Nature Reviews Microbiology. 2007;5:175-186

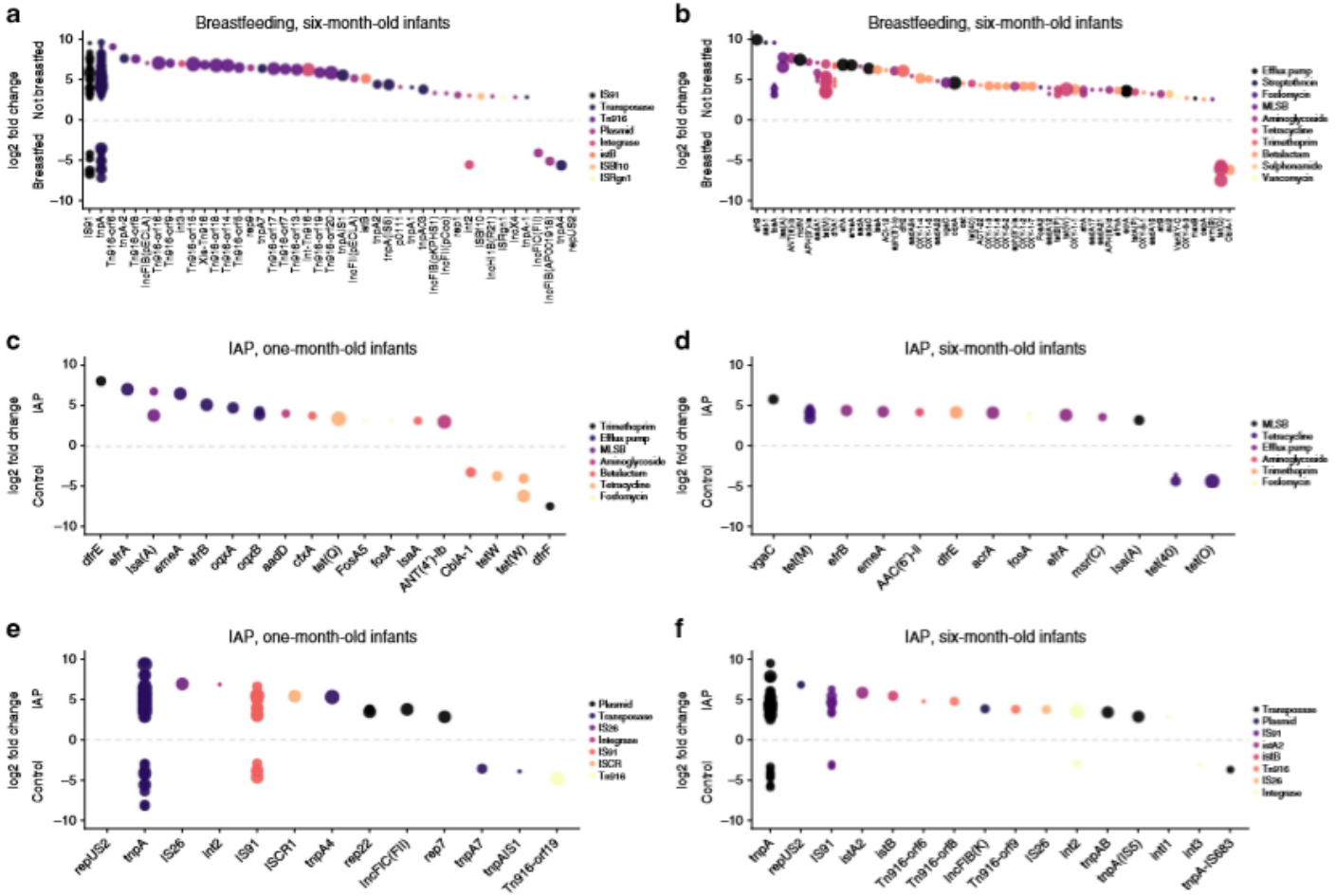
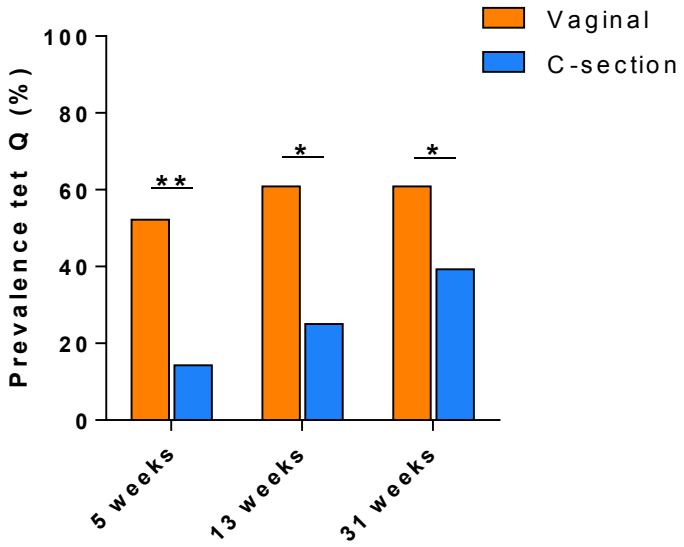
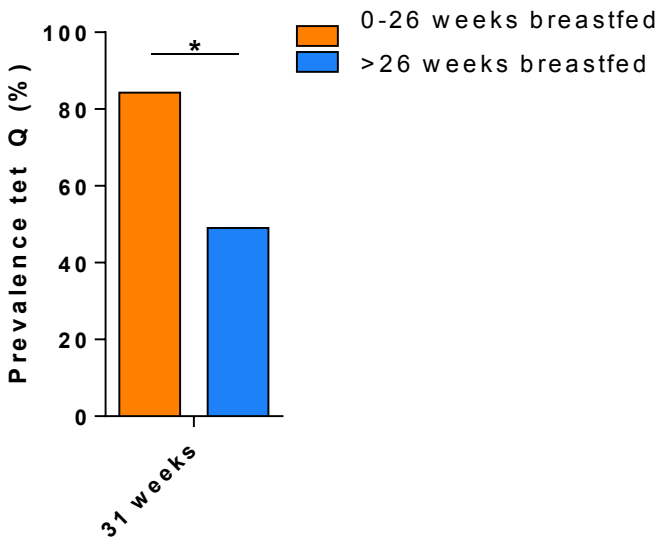
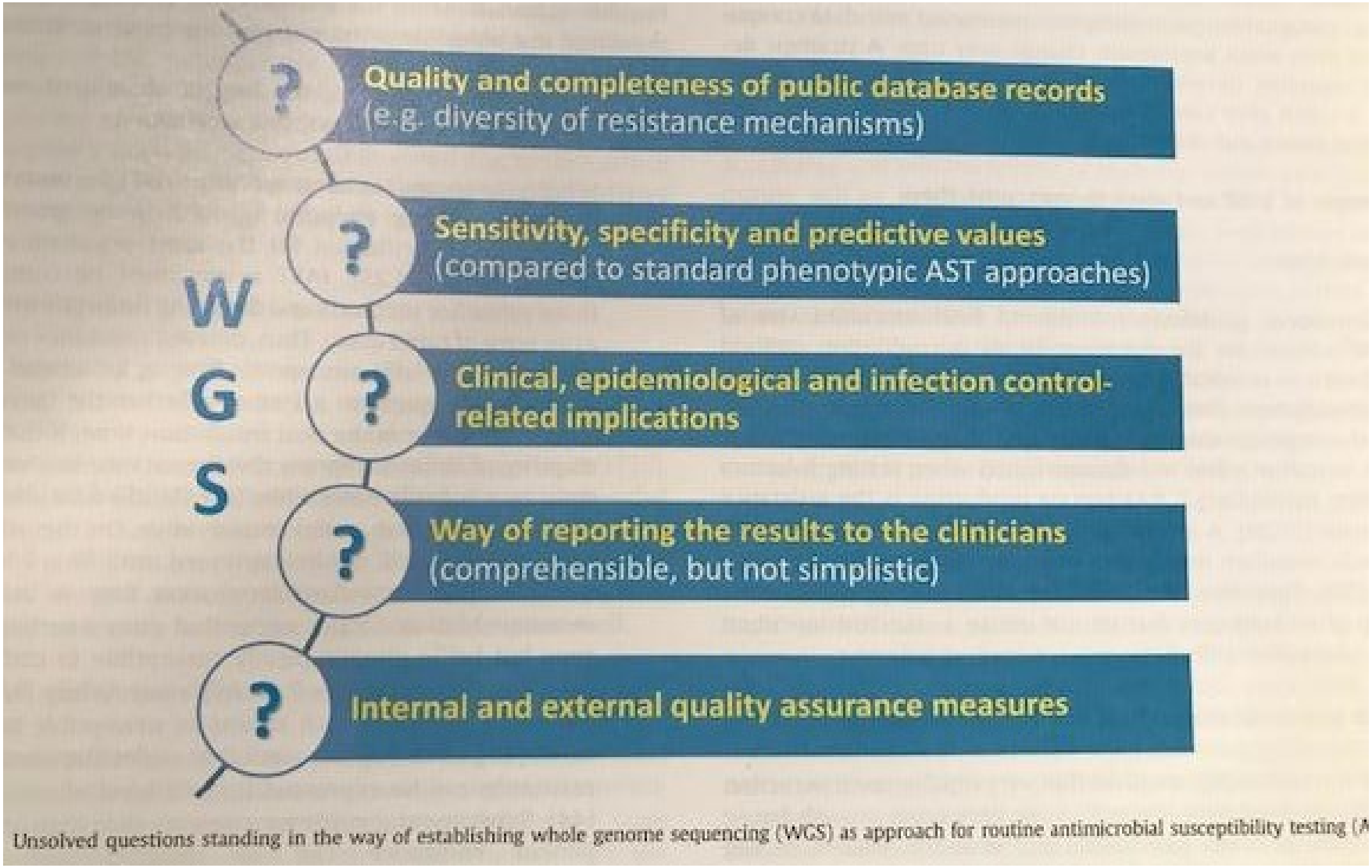


Fig. 6 Differentially abundant ARGs and MGEs in 6-month-old infants. **a** MGEs differentially abundant due to breastfeeding in 6-month-old infants compared to non-breastfed infants. **b** ARGs differentially abundant in breastfed infants at 6 months, **c** ARGs differentially abundant in 1-month-old infants due to IAP. **d** ARGs differentially abundant in 6-month-old infants due to IAP. **e** MGEs differentially abundant due to IAP in 1-month-old infants. **f** MGEs differentially abundant due to IAP in 6-month-old infants. Genes that have negative fold changes are more abundant in the non-breastfed and IAP groups. Sizes depict the number of samples each gene was found in ($n = 1$–10), color represents ARG or MGE class. The *y*-axis shows log2 fold changes and the *x*-axis denotes gene names

Mode of delivery and breastfeeding impact *tetQ* prevalence

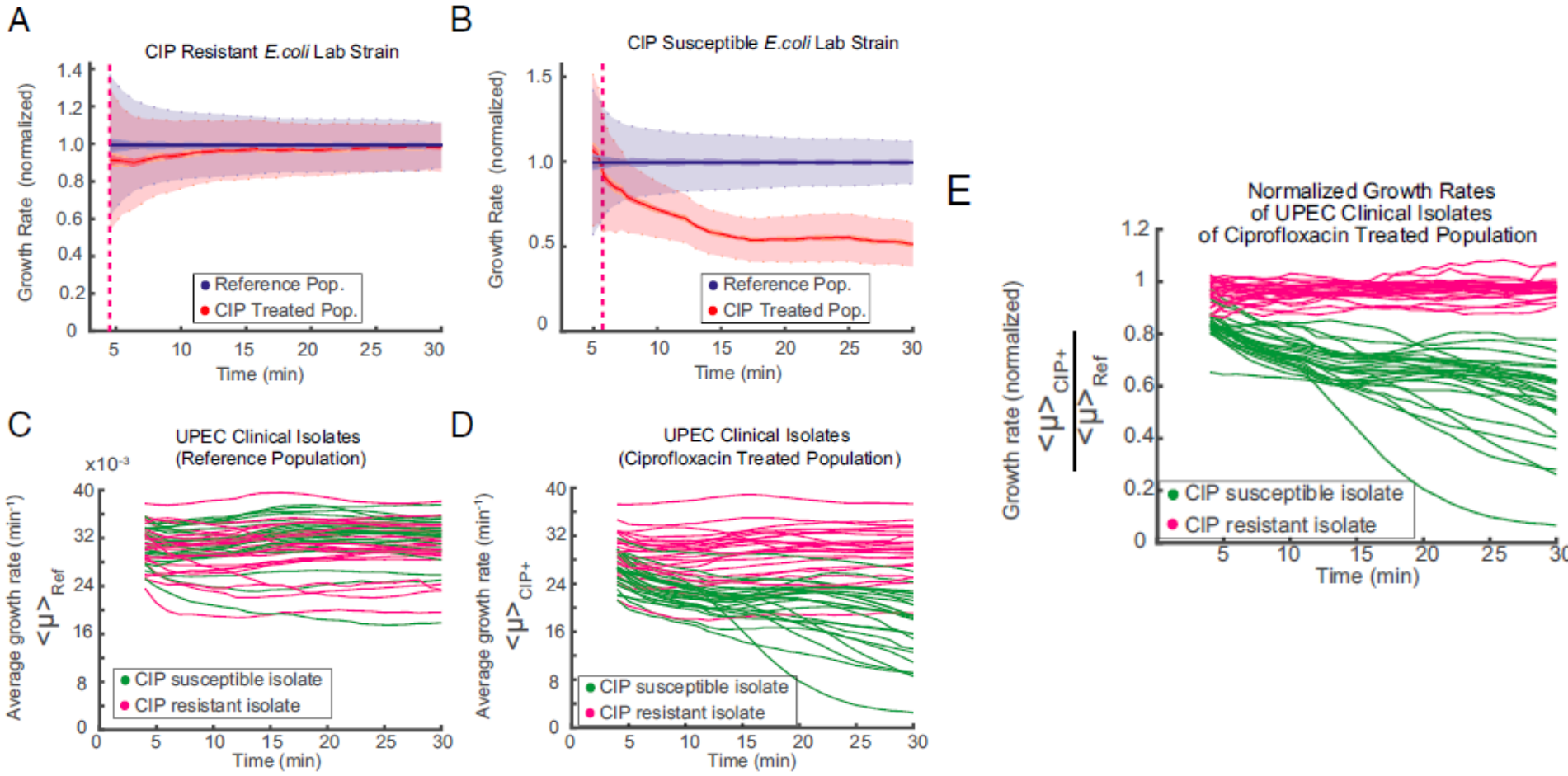






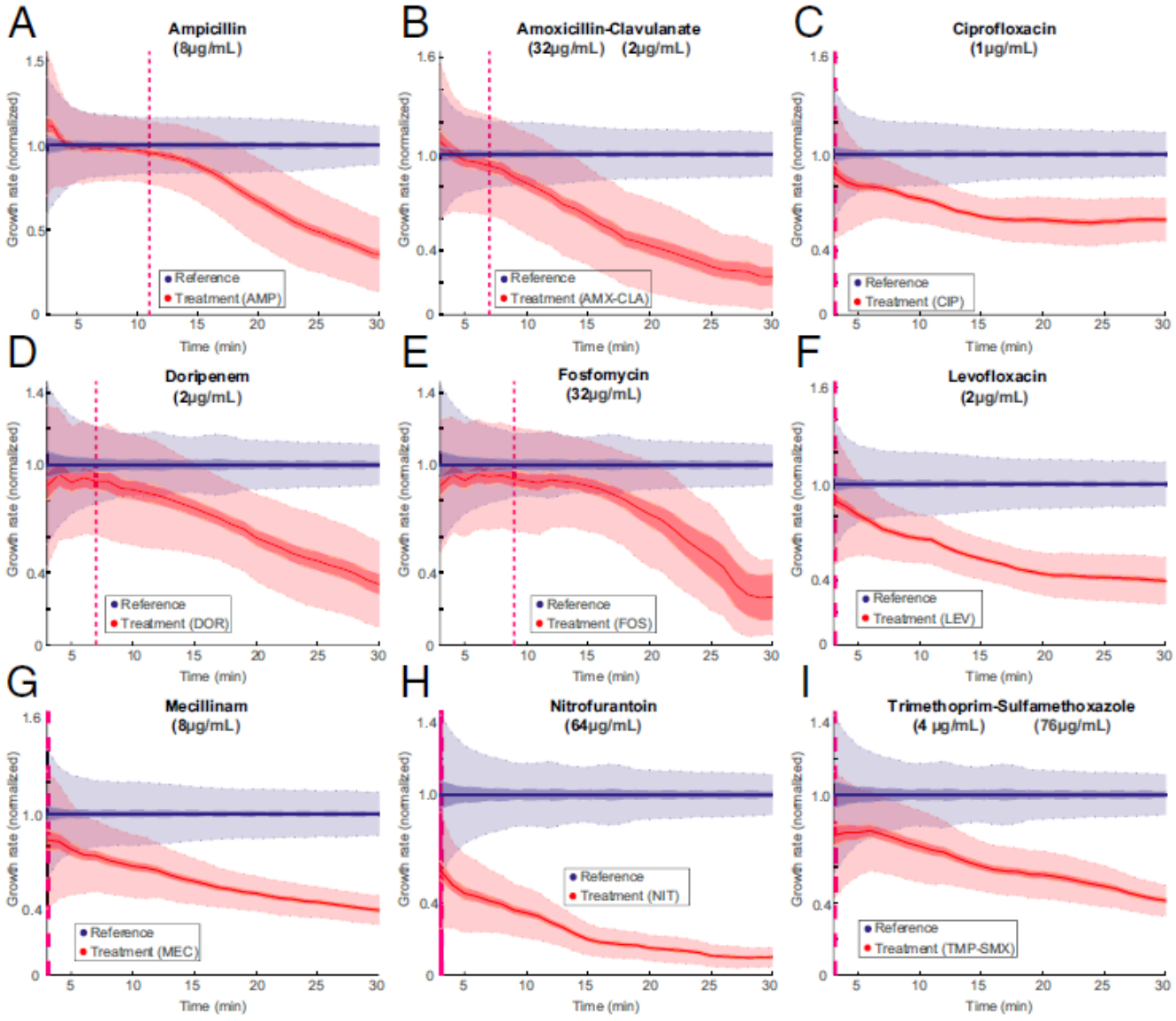
Unsolved questions standing in the way of establishing whole genome sequencing (WGS) as approach for routine antimicrobial susceptibility testing (A

Microculture: Fast detection of AMR in *E.coli*



(micro) Culture based

Microculture: Fast detection of AMR in *E.coli*



Current developments

Detection of AMR is changing (culture + molecular)

Detection of resistance become more complictaed due to Increase in diversity of resistance mechanisms

Molecular detection on clinical specimen is increasingly implemented

New technologies speed up detection of resistance

Role resistome will become more important (reservoir)

Miniaturisation of technologies & apparatus

Impact of novel molecular diagnostics on AMR

More rapid molecular detection of AMR will lead to optimized antibiotic treatment and thus less spread of AMR

Molecular diagnostics will give new insight in the resistome and thus AMR reservoir in clinical specimens

Molecular diagnostics will give better insight into transmission routes of specific genes and strains

MDx will give information on spread of mobile genetic elements

MDx will give us more time to develop new antibiotic strategies by decreasing the spread of AMR

Thanks for your attention

Christian von Wintersdorf
Lieke van Alphen
Petra Wolffs
John Penders
Paul Savelkoul