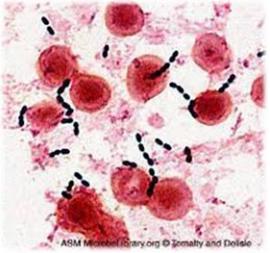


# Screening for linezolid resistant enterococci (LRE)

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*09/05/2023*

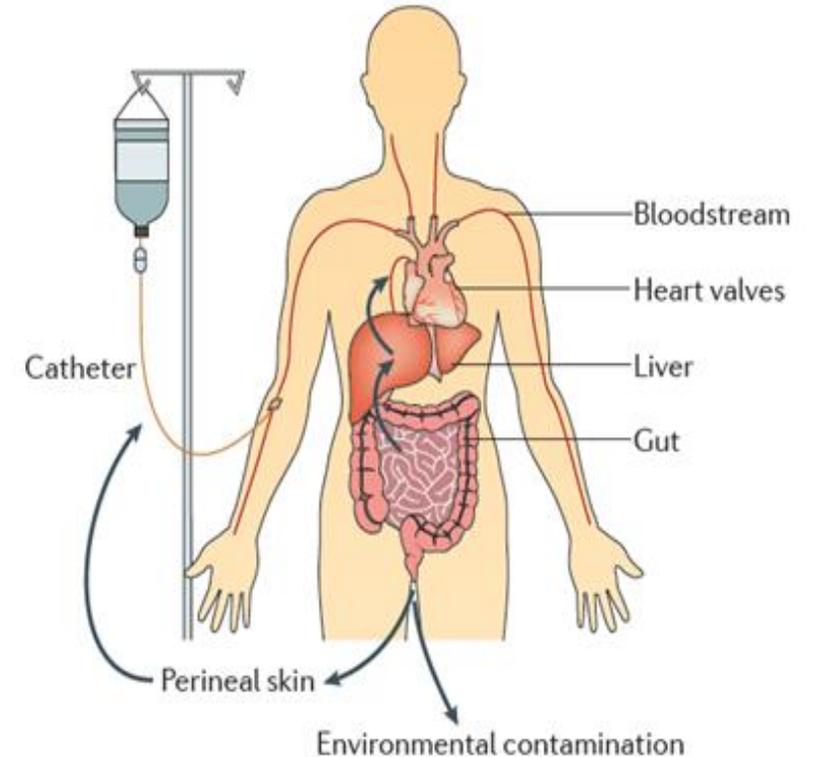
# Outline



- **AMR in *Enterococcus***
- **Oxazolidinone (linezolid) resistance mechanisms**
- **Antimicrobial susceptibility testing (AST)**
  - Disc diffusion, gradient tests and broth microdilution (BMD)
- **Direct screening for linezolid resistant enterococci (LRE)**
- **Summary**

# The clinical importance of enterococci

- Important **opportunistic pathogen**:
  - Urinary tract infections (UTI)
  - Blood stream infections (BSI) and endocarditis
  - Intraabdominal infections
  - Risk: immunocompromised, abdominal surgery, elderly
- Antibiotic treatment - limited due to **intrinsic resistance**
- **Colonization pressure** – risk for infections
- **Easily acquire resistance**



# Development of multidrug resistance

## Inherent resistance

- Cephalosporins
- Sulphonamides
- Lincosamides
- Low levels of aminoglycosides
- Streptogramins: quinupristin-dalfopristin (*E. faecalis*)

## Acquired resistance

- Ampicillin (*E. faecium*)
- High levels of aminoglycosides
- Vancomycin
- Fluoroquinolones
- Oxazolidinones (linezolid)
- Quinopristine-dalfopristin (*E. faecium*)
- Daptomycin, eravacycline/tigecycline

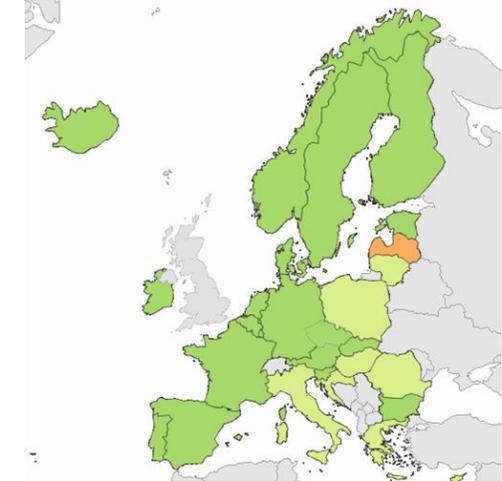
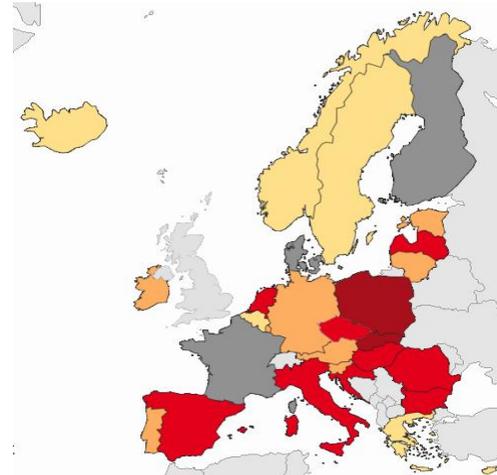
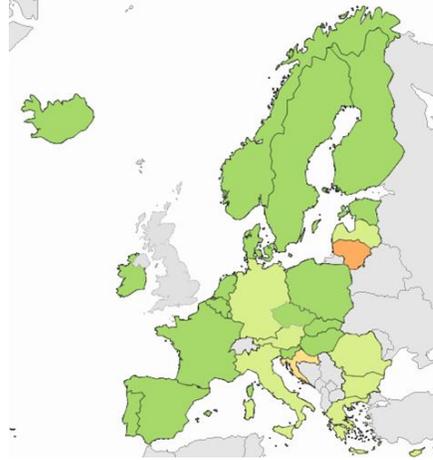
# Resistance *E. faecalis* versus *E. faecium* 2021

Aminopenicillins

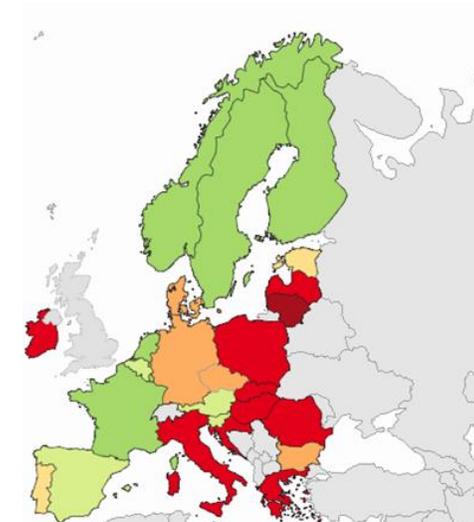
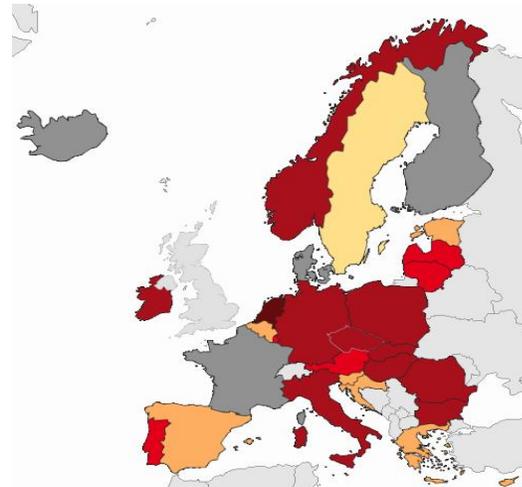
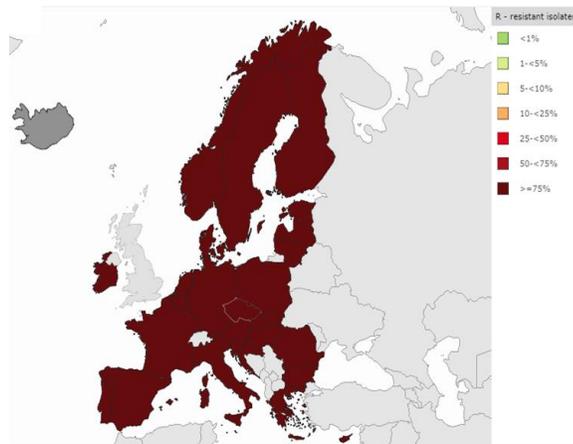
High-level gentamicin

Vancomycin

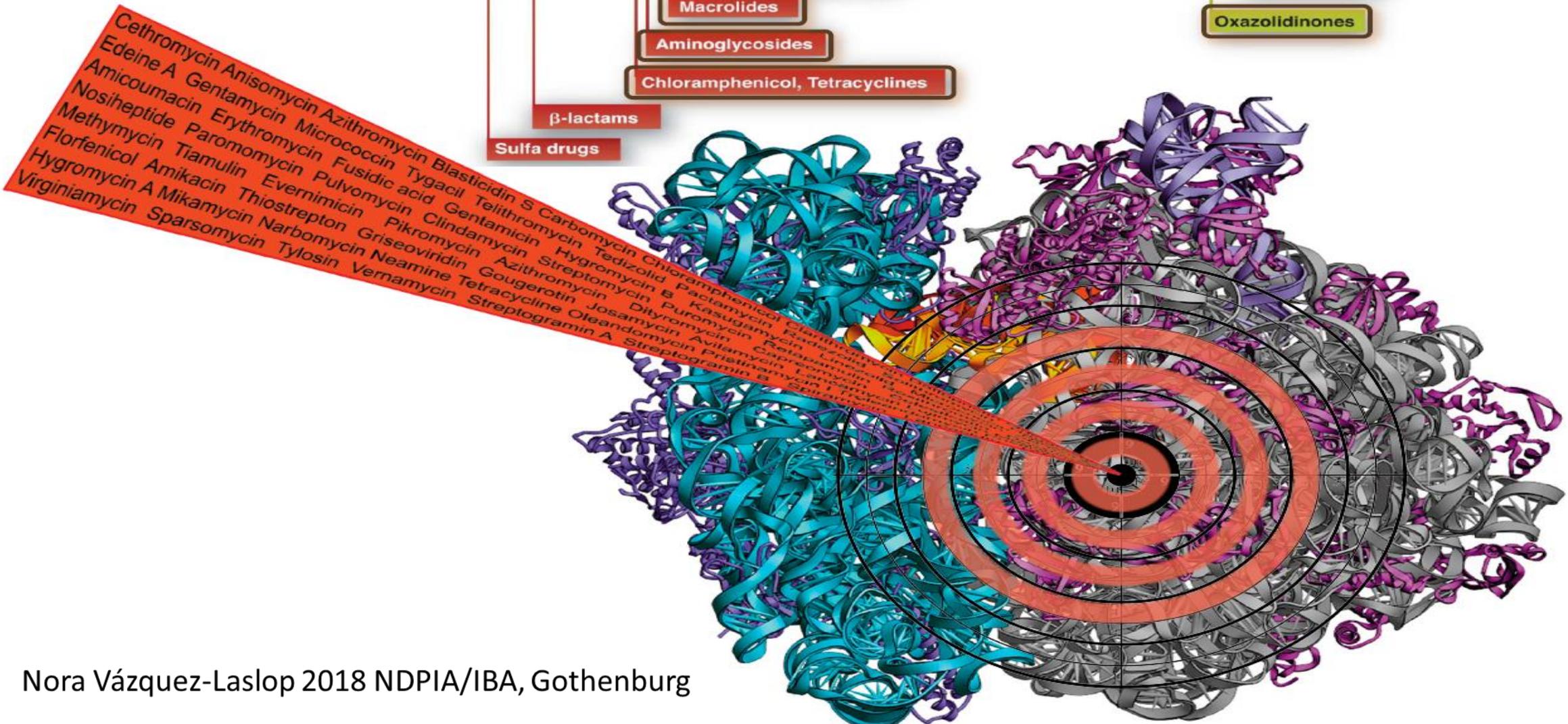
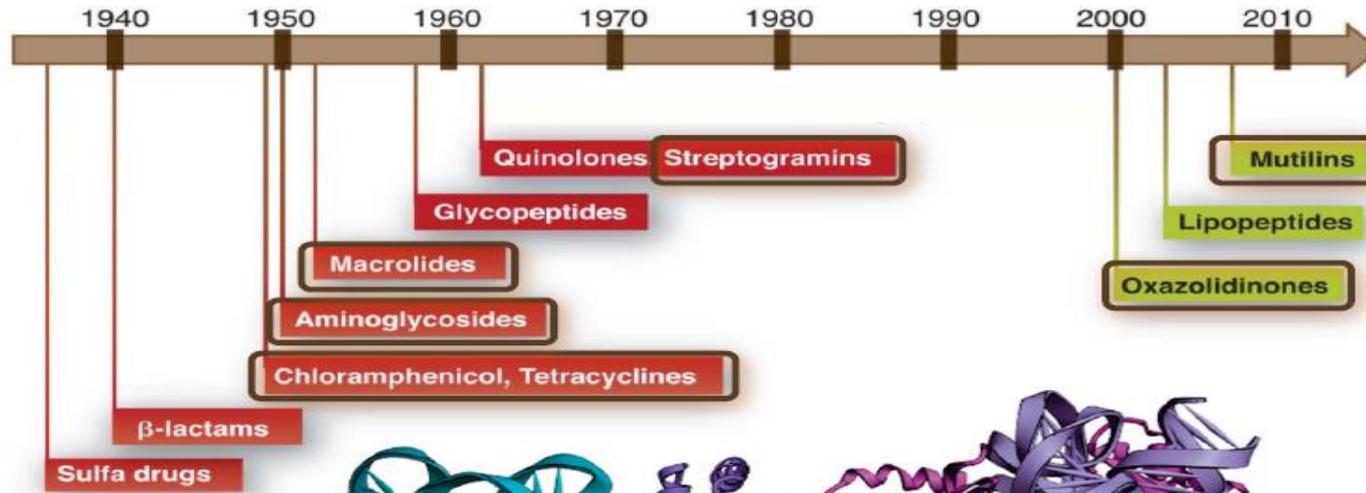
*E. faecalis*

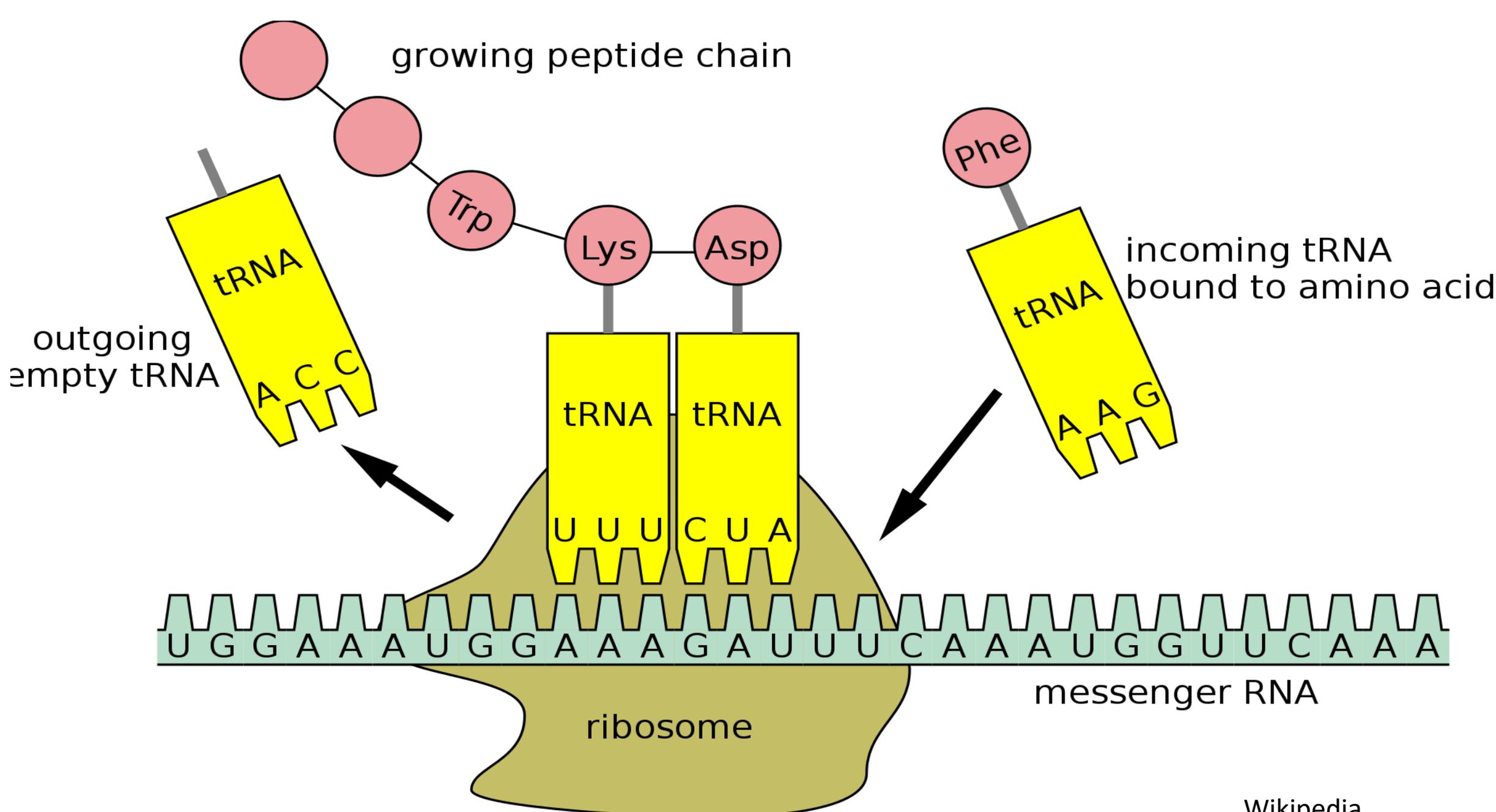


*E. faecium*

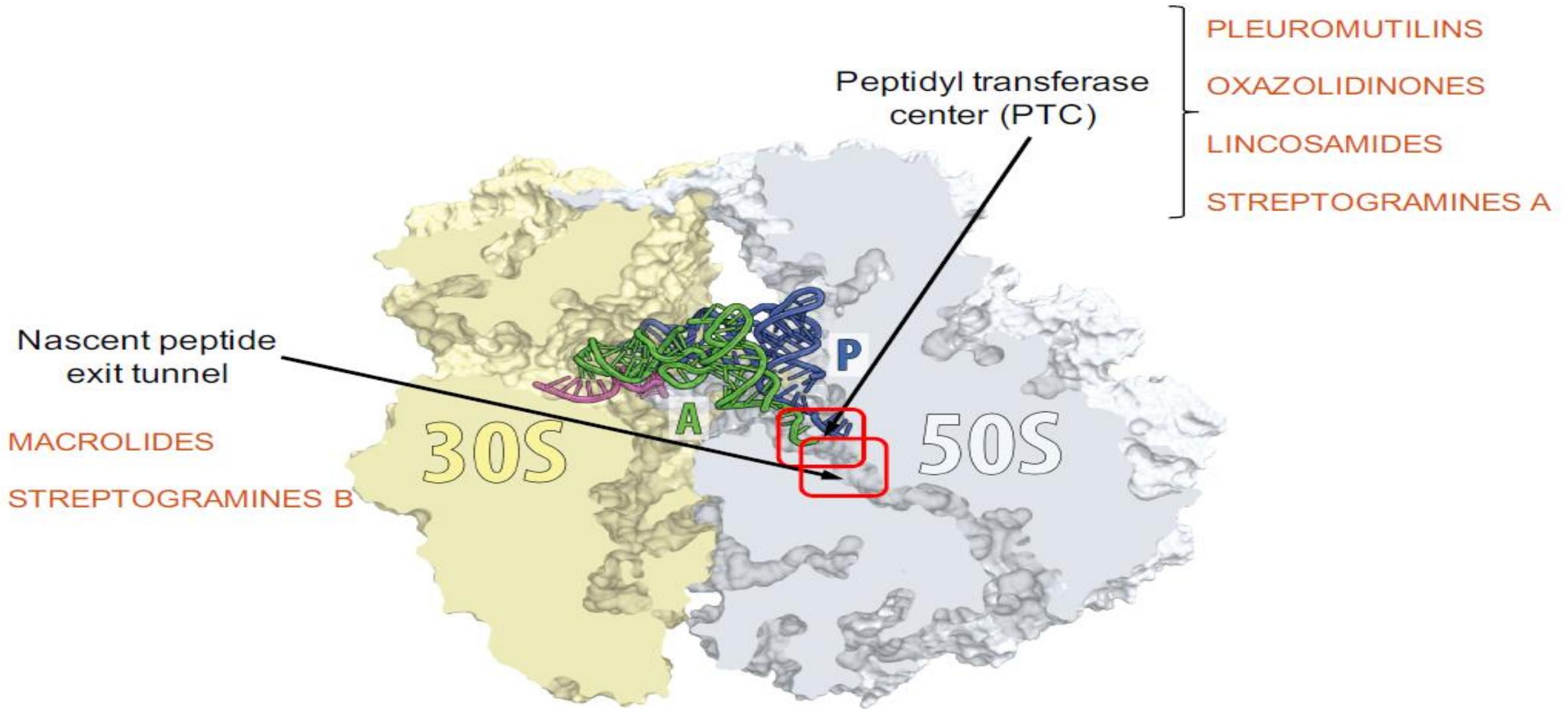


# Ribosome is a preferred antibiotic target





# Peptide Synthesis



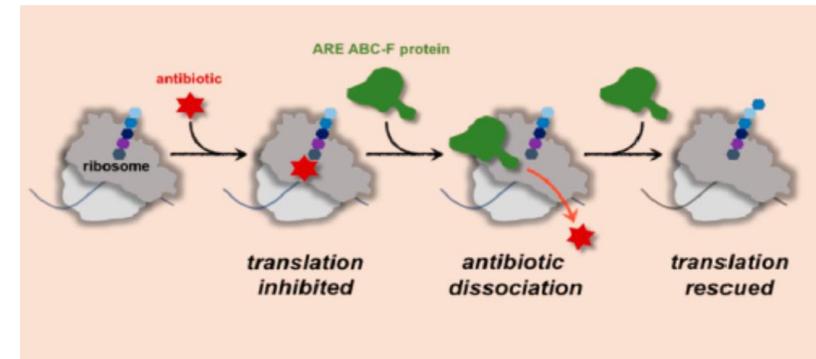
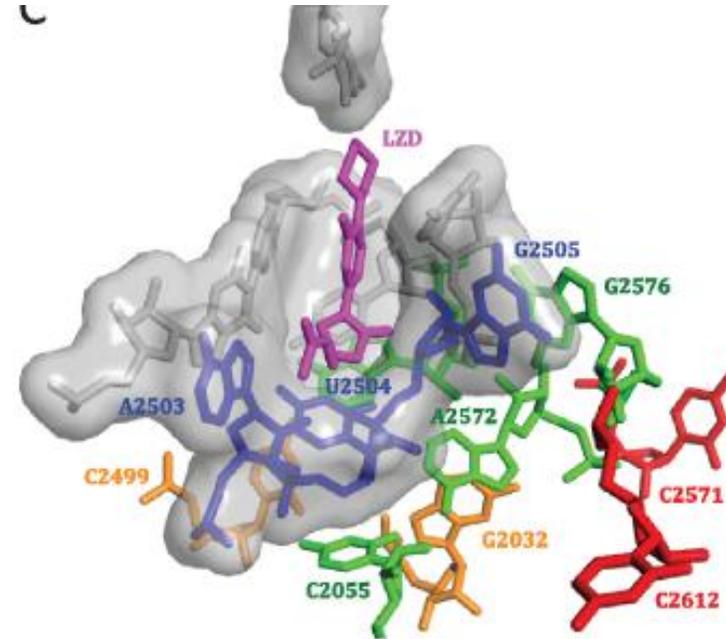
# Oxazolidinone resistance mechanisms

## Mutations in ribosome

- Mainly **G2576U** mutation in 23S rRNA V domain
- Related to oxazolidinone exposure
  - R: linezolid, tedizolid
- Variable linezolid MIC - depend on number of 23S rRNA copies gained mutation
- Ribosomal protein mutations also identified

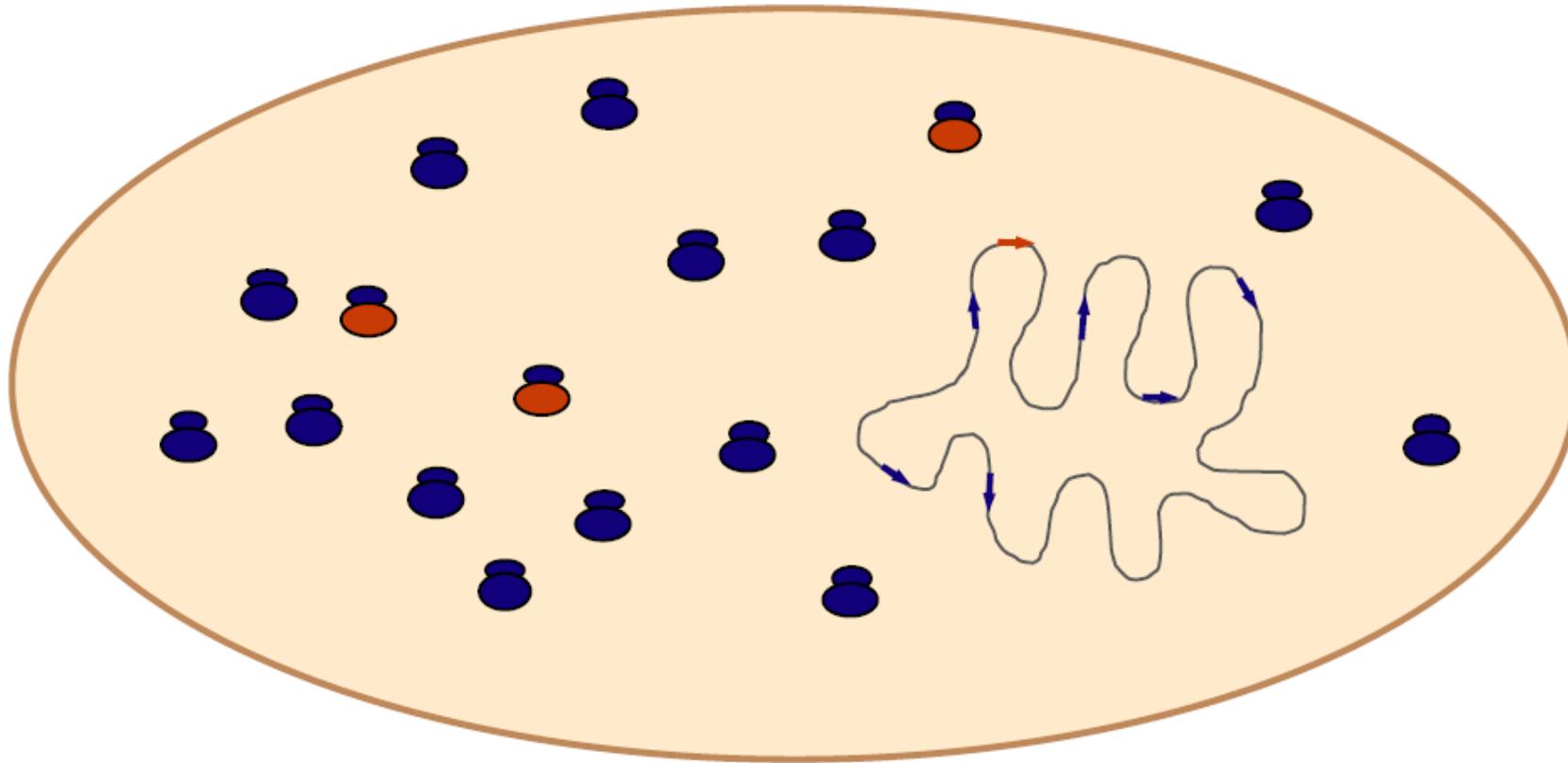
## Transferable ribosomal protection

- ***optrA*, *poxtA* and *poxtA-like***
  - ABC-F proteins: ATP-binding cassette F-protein subfamily
  - R: linezolid, tedizolid, phenicols
  - R/S: tetracycline
- **Cfr (rRNA methyltransferase):** R to linezolid?!
- Linezolid MIC can be around breakpoint

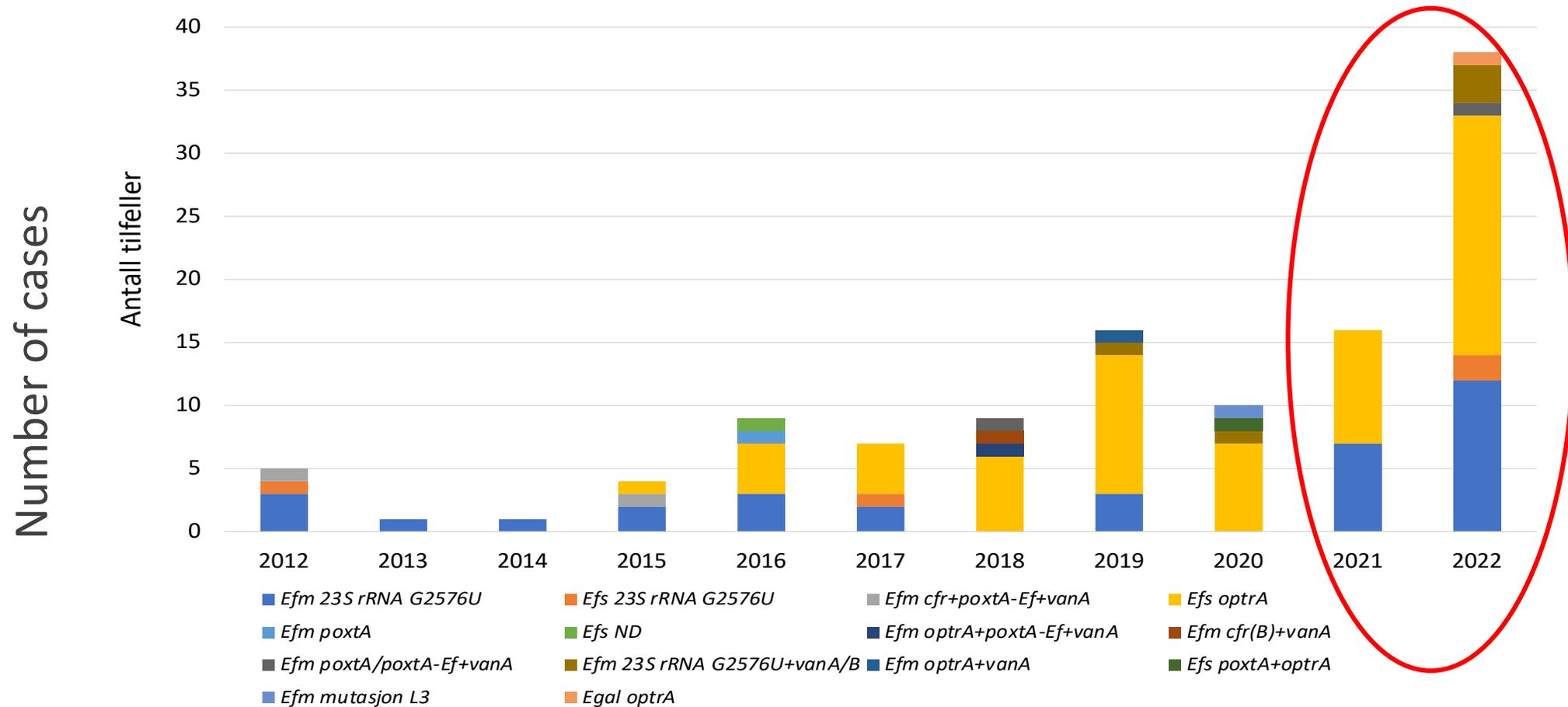


A single spontaneous resistance mutations is usually recessive and does not confer sufficient level of antibiotic resistance

## Mutations in 23S rRNA



# Linezolid resistance mechanisms - Norway



# AST - enterococci

- **Intrinsic resistance:**

- Cephalosporins, sulphonamides, lincosamides,
- Low-level resistance to aminoglycosides

- **First line drugs:**

- Ampicillin, gentamicin, vancomycin
- Uncomplicated UTI: nitrofurantoin (*Efs*), ciprofloxacin, trimethoprim

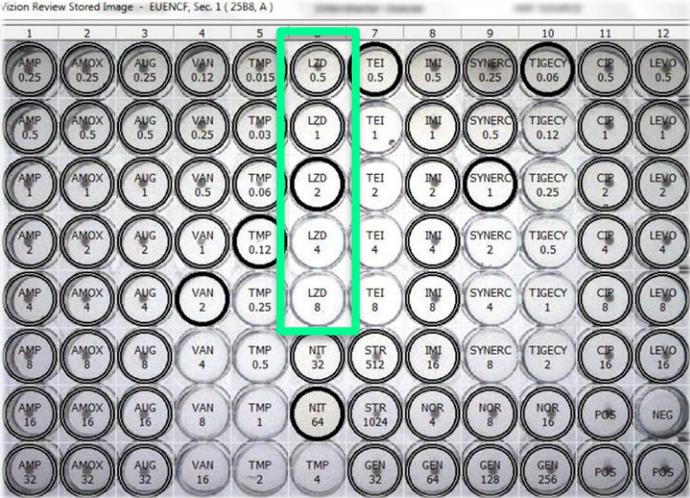
- **Second line drugs:**

- linezolid, tigecycline, daptomycin, eravacycline, lipoglycopeptides



# Phenotypic AST linezolid

**Gold standard**  
Broth micro dilution



**Gradient strips**



**EUCAST disk diffusion**



# Performance of the EUCAST disc diffusion method and gradient tests in detection of linezolid susceptibility in *Enterococcus faecalis* and *Enterococcus faecium* (P3074 ECCMID 2021)

ning av antibiotikaresistens

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<sup>3</sup>Research Group for Host-Microbe Interactions, UiT the Arctic University of Norway, Tromsø, Norway.

**Table 1. Bacterial strains and their linezolid resistance determinants.**

	LRE (n=48*)				LSE (n=34)
	<i>optrA</i>	<i>poxtA/poxtA-like</i>	<i>cfr</i>	23SrRNA G2576U	
<i>Efm</i>	2	5	3	14	21
<i>Efs</i>	26	0	0	2	13

\* Four *Efm* strains carried two different linezolid resistance determinants; *cfr+poxtA-like* (n=3) and *optrA+poxtA-like* (n=1).

- **Material**

- 82 whole genome sequenced *E. faecalis* (n=41) and *E. faecium* (n=41). LRE (n=48), LSE (n=34)
- Examined blindly at EDL

- **Methods**

- Resistance was defined by broth microdilution (BMD; Sensititre) and/or genotype
- EUCAST disk diffusion method (Oxoid and BD)
- Gradient tests: MTS (Liofilchem) and Etest (bioMérieux)

# Conclusions P3074 ECCMID 2021

## *Conclusions*

- ✓ The EUCAST DD method is a robust method for determining susceptibility to linezolid for *Efs* and *Efm*.
- ✓ MIC-values of 4 mg/L and DD inhibition zones of 20 mm might represent an area of technical uncertainty.
- ✓ The performance of linezolid gradient test for MIC-determination needs to be monitored carefully.

# Direct screening for LRE

- «SuperLinezolid medium»
- **BHI-agar with:**
  - Linezolid 1.5 mg/L + Aztreonam 2 mg/L
  - Colistin sulfate 15 mg/L
  - Amphotericin B 5 mg/L
- **Validation**
  - Single G+ strains (n=37; R =17 MIC ≥ 8 mg/L)
    - *E. faecium* (n=1), *E. faecalis* (n=7)
  - Gram-negatives (n=11)
  - Spiked stools (n=3; 8 R-strains)
  - Sensitivity/specificity cut-off value
    - $1 \times 10^3$  CFU/ml
- **Results:**
  - 24-hour
    - 3/17 R-strains negative
  - 48 hours
    - All R strain positive
    - 1/20 S-strains positive
    - All R-strains in spiked stool – positive
    - All Gram negative: negative

# Direct screening for LRE

- **CHROMagar™ LIN-R**
  - Based upon «SuperLinezolid medium
- **Strains** - extended validation (DMID 2021)
  - Enterococci (n=47; Lin-R n=35): AST by BMD (BD)
  - 100 sensitivity and specificity
- **Rectal samples** – German multicenter (ECCMID 2023)
  - Samples (n=11.477) and centers (n=12)
  - LRE prevalence 0.9%
  - LRE confirmation by BMD (+ genotyping)
  - High false positive rates
    - Average 57% (range 0 – 93%)
  - High rate of genotype R/phenotype S
    - *E. faecium* (n=21/101), *E. faecalis* (n=10/50)

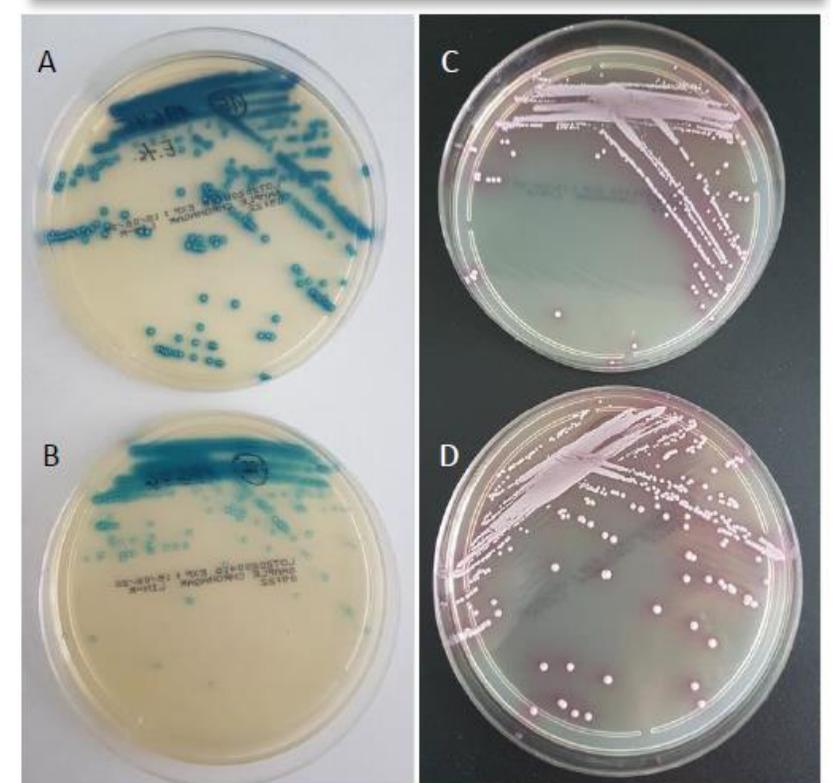


Figure 1. Growth behaviour of linezolid-resistant isolates of *E. faecalis* (A), *E. faecium* (B), and *S. epidermidis* (C,D) after 48 h.

# Summary

- **Linezolid resistance mechanisms**
  - Transferable resistance encoded by *optrA*, *poxA*
    - Polyclonal, *E. faecalis* and other species
  - Chromosomal mutations
    - *E. faecium*; Related to linezolid selection
  - Linezolid MICs often close to the R-breakpoint
- **AST**
  - Clinical breakpoints
    - Potential need for an ATU zone (20 mm) and MIC (4 mg/L)
  - Transferable resistance determinants challenge current breakpoints
- **Direct LRE screening**
  - One commercial direct screening method
    - Needs further validation - ongoing

