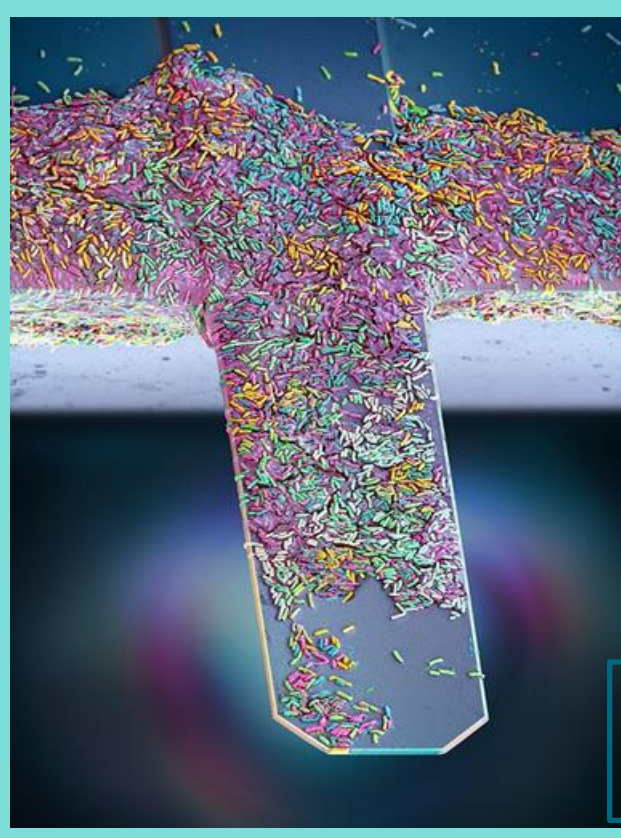


A rapid and high performing antibiotic susceptibility test for meropenem based on bacterial nanomotions

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Cantilever with attached *E. coli*

Infections caused by multidrug resistant bacteria require the use of **last-resort antibiotics** such as **meropenem**, racing the spread of resistances to carbapenems. *E. coli* and *K. pneumoniae* are the most common Gram-negative bacteria causing **bloodstream infections**. *K. pneumoniae* already presents over **10% of resistance to meropenem** in south and eastern Europe countries^[1].

AST methods, combined with carbapenemases detection, are used for the diagnosis of carbapenem resistant bacteria. However, there is a need for faster phenotypic methods for the accurate classification of phenotypes and determination of an informed treatment.

Nanomotion Technology

- **Rapid, Phenotypic** and **Growth independent** method.
- Based on a **real-time** recording of **bacterial vibrations** (nanomotions) caused by **cellular metabolic activity**.
- **Viable bacteria** are **attached to a cantilever**, causing **cantilever deflections** (figure 1).
- **Signal**: High frequency measurements (60kHz) are based on an optical readout involving a SLED and photodetector (figure 2).
- **Machine-learning** based **classification algorithm** using **signal parameters** from the **recording**.

Figure 1. Principle

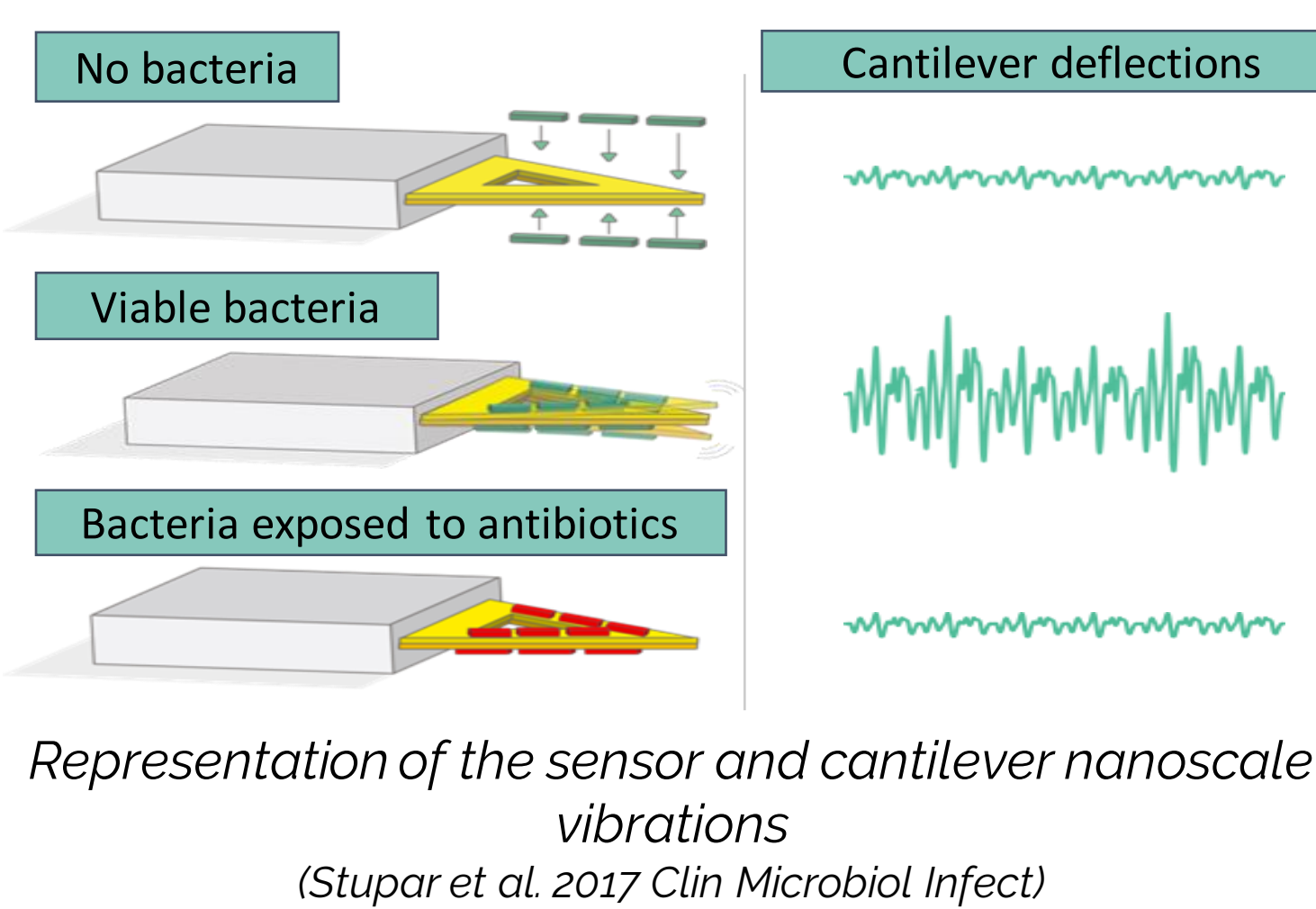
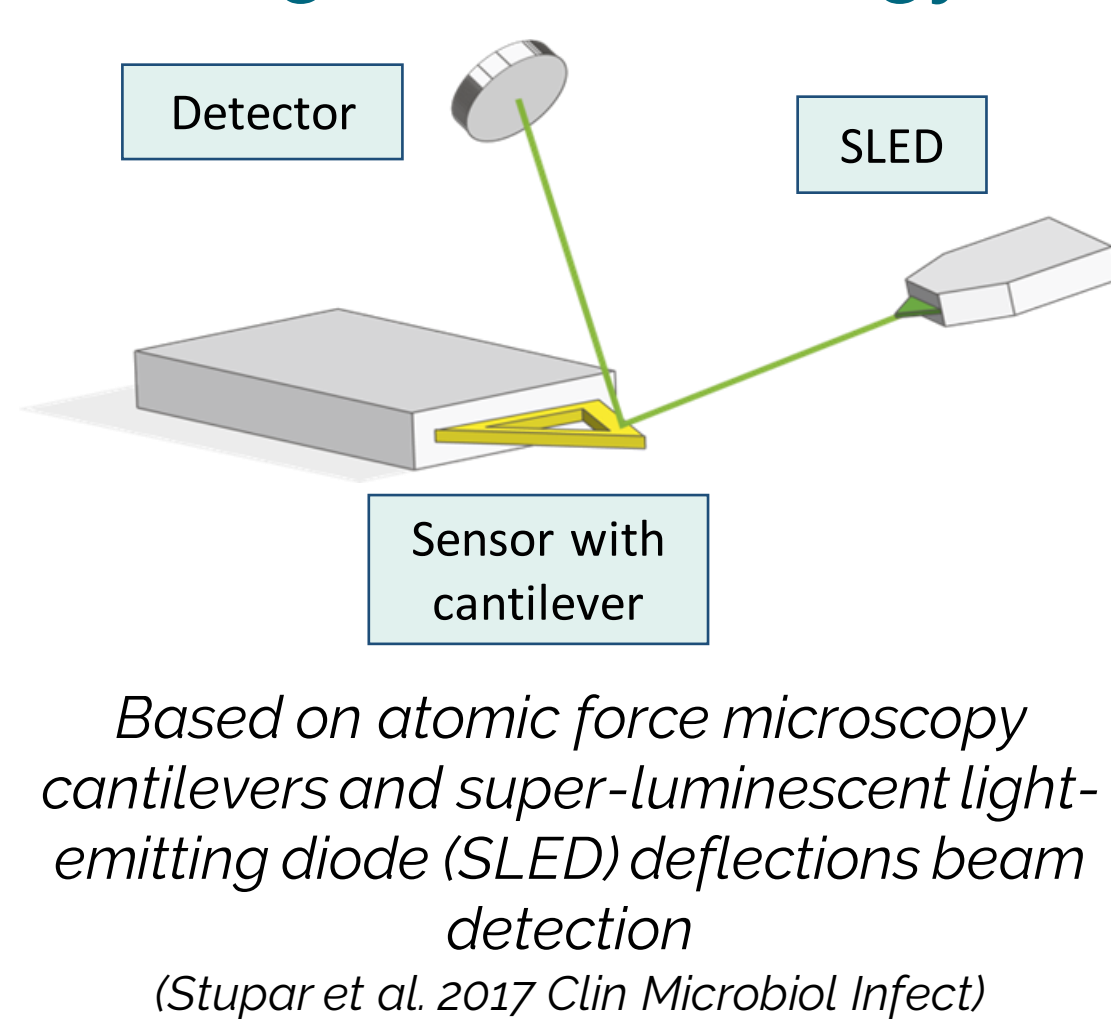
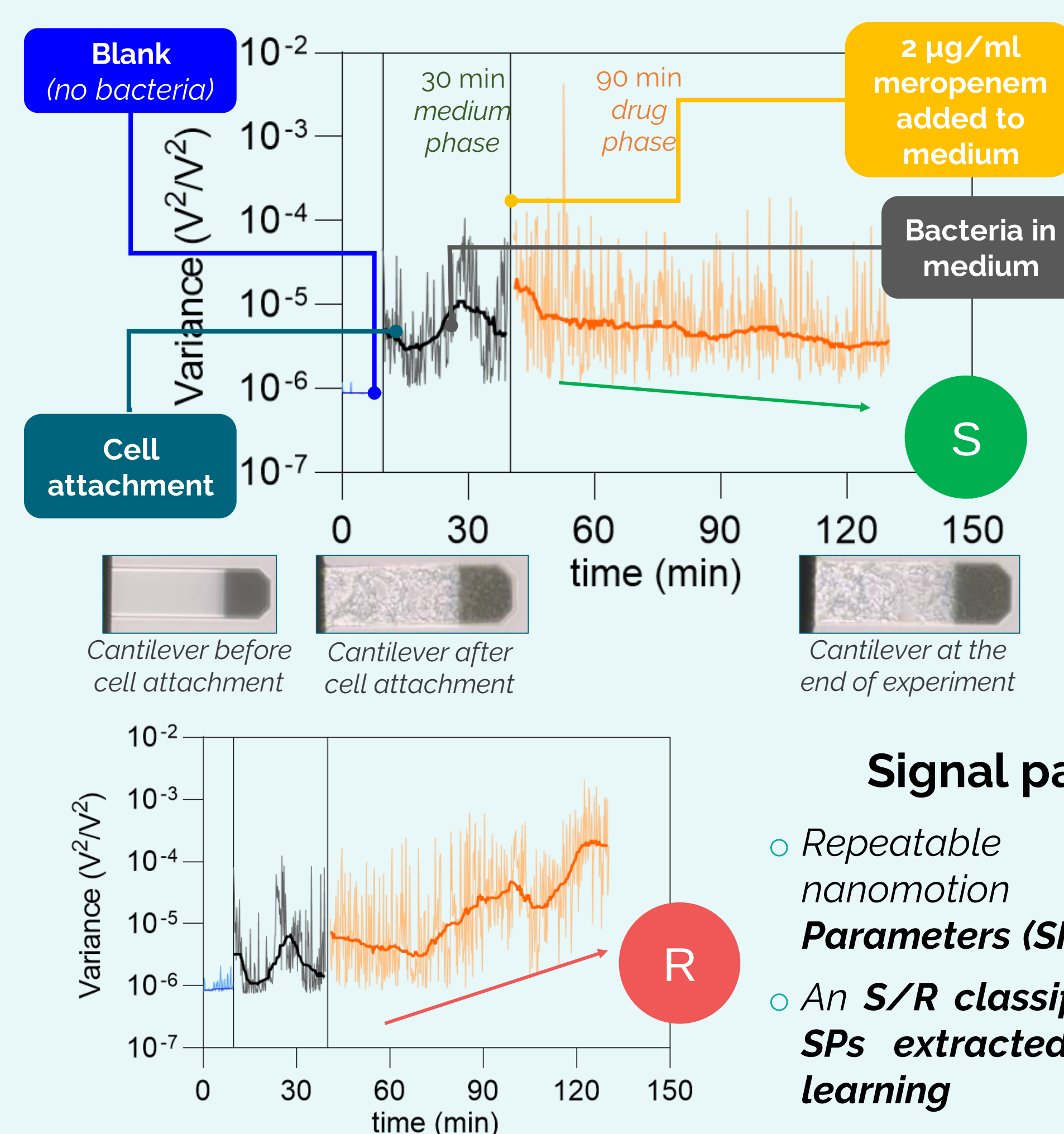


Figure 2. Technology



AST set-up and results in 2 hours



- The **variance (V_2/V_2)** represents the oscillation of the cantilever. It is calculated as its deflection (detected in volts) divided by the total voltage detected.
- A few hundred bacteria on the cantilever suffice.

Signal parameters extraction

- Repeatable characteristics present in nanomotion are defined as "**Signal Parameters (SPs)**" like the slope
- An **S/R classification algorithm** is based on **SPs extracted and selected via machine learning**

Nanomotion technology platform + assisted machine learning → Accurate AST in 2h



Figure 3. Hardware

The **PHENOTECH RESEARCH** device developed to measure **nanoscale vibrations** as an indicator for **cellular viability**.

Chamber holding the Sensor.
Can be filled with liquid (e.g., growth medium, etc.)



Sensor with microscopically small cantilever on tip

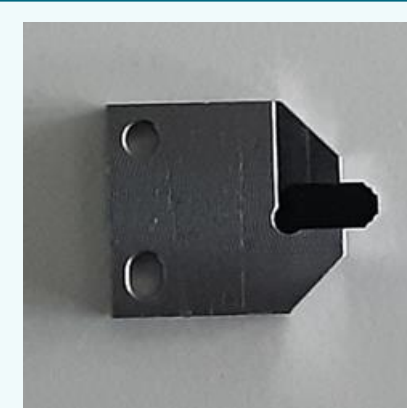
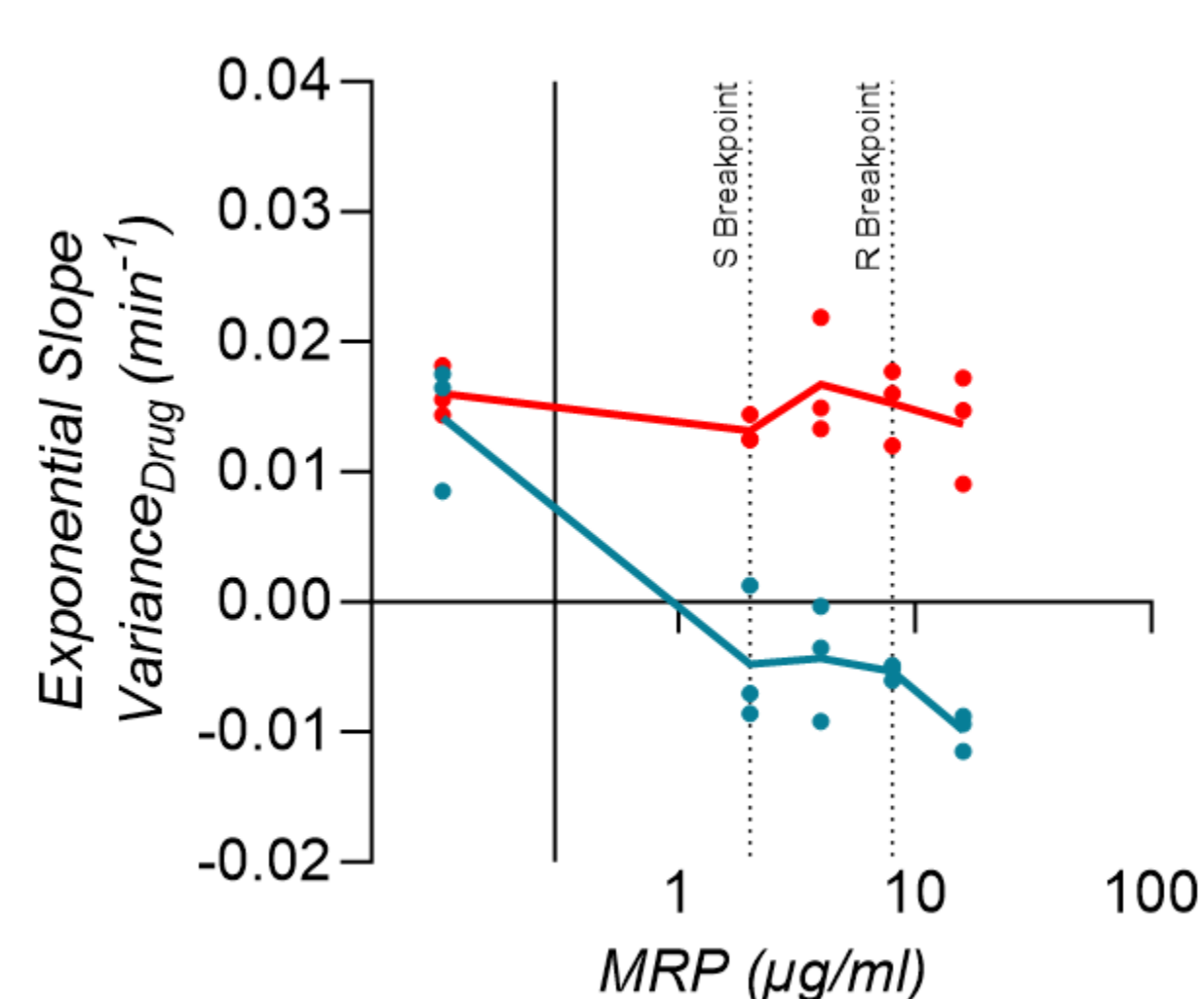


Figure 4. Dose response



- **ATCC-25922** (MIC < 0.25 µg/ml)
- **NARA5005** (MIC = 128 µg/ml)
- Concentrations of meropenem:
→ 0, 2, 4, 8, 16 µg/ml
- MIC breakpoints for meropenem:
→ Susceptible ≤ 2 µg/ml
→ Resistant > 8 µg/ml

- **Slope** (signal parameter) of the **90 min nanomotion** after addition of **meropenem** of the recording
- **R strains** present a constant positive slope with all concentrations tested
- **S strains** show a decrease in the slope with increasing concentrations

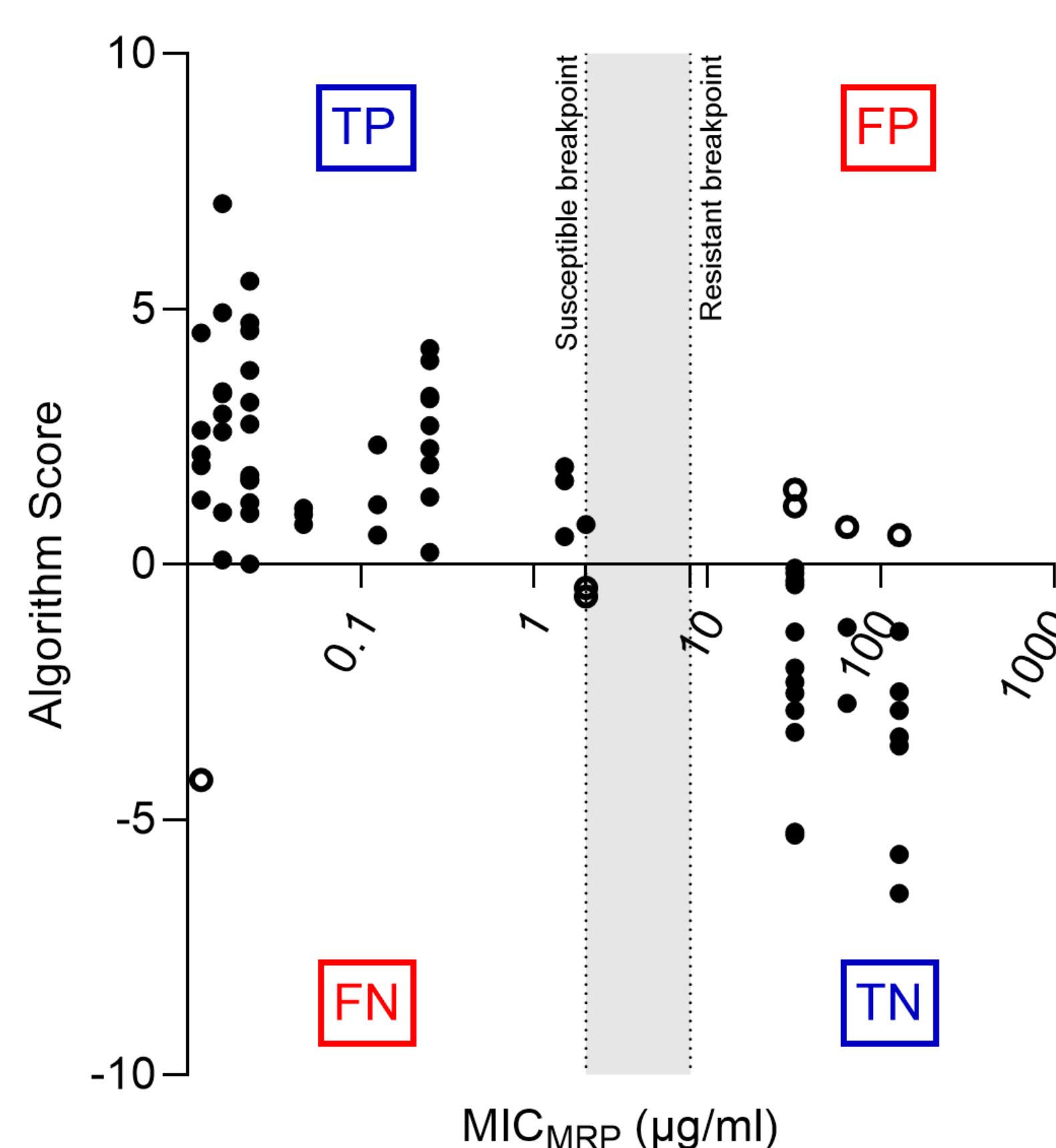
Figure 5. Beta-lactams algorithm

Training: Several signal parameters are required for phenotype classification

E. coli and *K. pneumoniae* reference strains and clinical isolates are used to develop antibiotic or antibiotic class dependant classification algorithms:

Pathogen	Antibiotic	Antibiotic class	No. experiments Training (No. strains)	Acc. Training (%)	Sen. Training (%)	Sp. Training (%)
<i>E. coli</i> + <i>K. pneumoniae</i>	ceftriaxone (CRO)	cephalosporin	847 (179)	94.7	93.6	95.7
	ceftazidime-avibactam (Zavicefta)	cephalosporin + beta-lactamase inhibitor		95.1	92.9	98.0
	piperacillin-tazobactam (TZP)	penicillin + beta-lactamase inhibitor		96.3	96.6	96.0
	Meropenem (MRP)	carbapenem		90.5	93.6	85.2
	CRO, Zavicefta, TZP, MRP	4 combined		94.0	94.3	93.7

Meropenem: Test concentration = 2 µg/mL



Experiment level	
n	74
TP	44
TN	23
FP	4
FN	3
accuracy (%)	90.5
sensitivity (%)	93.6
specificity (%)	85.2

Sample level (voting)	
n	25
TP	15
TN	9
FP	0
FN	1
accuracy (%)	96.0
sensitivity (%)	93.7
specificity (%)	100