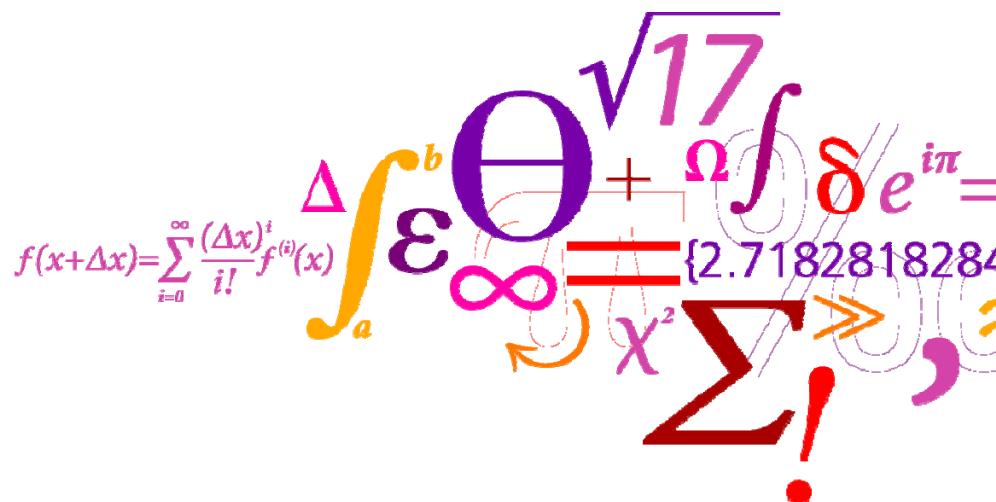


EURL-AR training course on genotypic characterization of antimicrobial-resistant bacteria

8 November 2011
Kgs. Lyngby, Denmark

DTU Food
National Food Institute



Yesterday...

Lab theme 1:

Detection and characterization of AR determinants of public health relevance (beta-lactamase-encoding genes & PMQR)

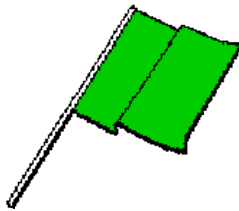


WHEN

WHAT

WHY

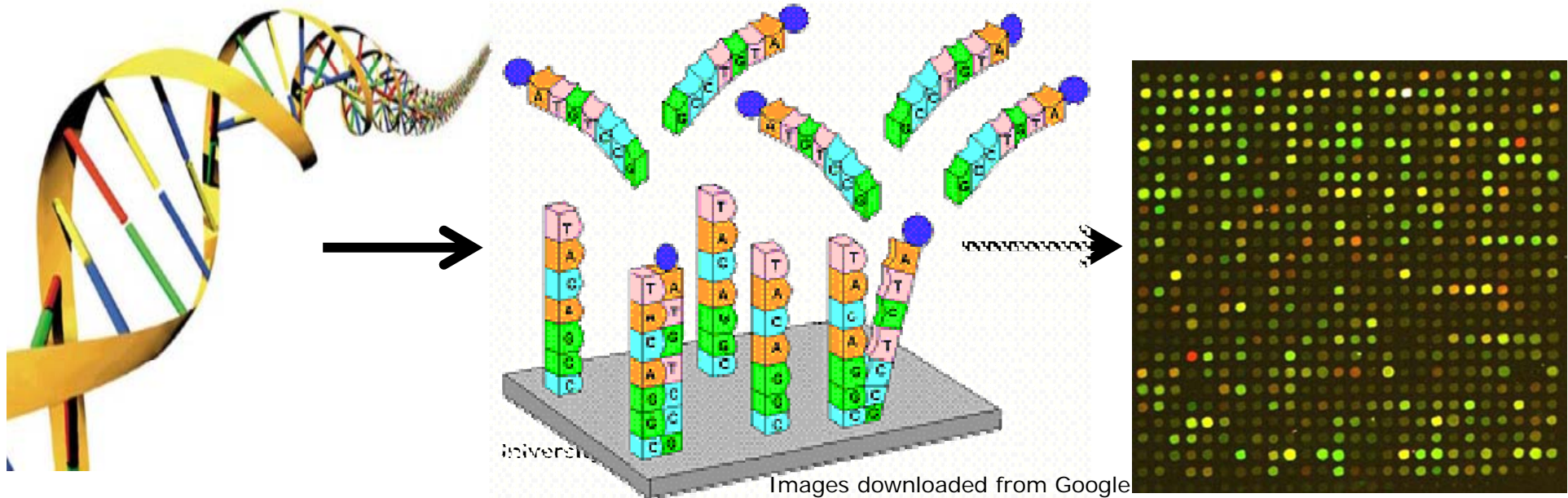
MONDAY



Microarray

For simultaneous detection of multiple AR genes

DNA hybridization-based genotyping method



Lab theme 2: MRSA surveillance



WHEN

MONDAY

WHAT

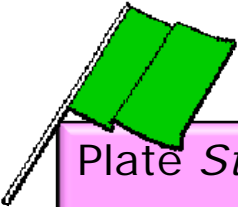
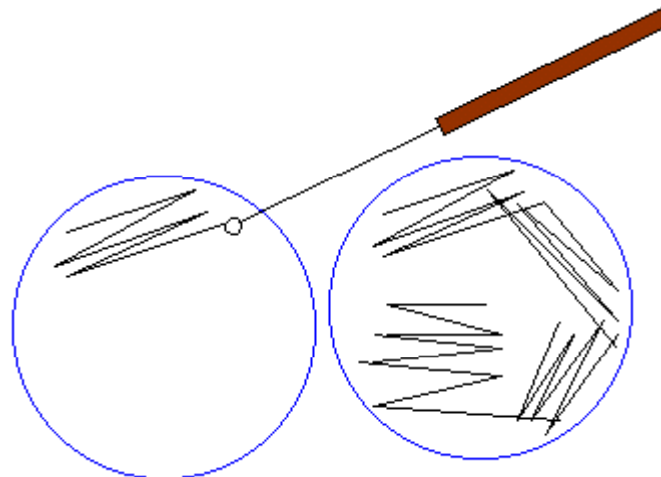


Plate *Staphylococcus aureus* isolates on selective agar plates

WHY

To isolate suspect MRSA



Images downloaded from Google

...and today:

- **Lab theme 1:**

1) Analyze microarray results:

Which AR genes are present in the isolates given to your group?

Is there correspondance between phenotype (MIC values) and genotype (microarray results)?

2) Remember this week's situation: we are doing a pilot study to genotype bacteria showing an antimicrobial resistance phenotype of public health interest:

Based on the results you have so far, which genes would you target for further characterization?

We will discuss these issues with each group in a short while

Lab theme 1: Detection and characterization of AR determinants of public health relevance (beta-lactamase-encoding genes & PMQR)



WHEN

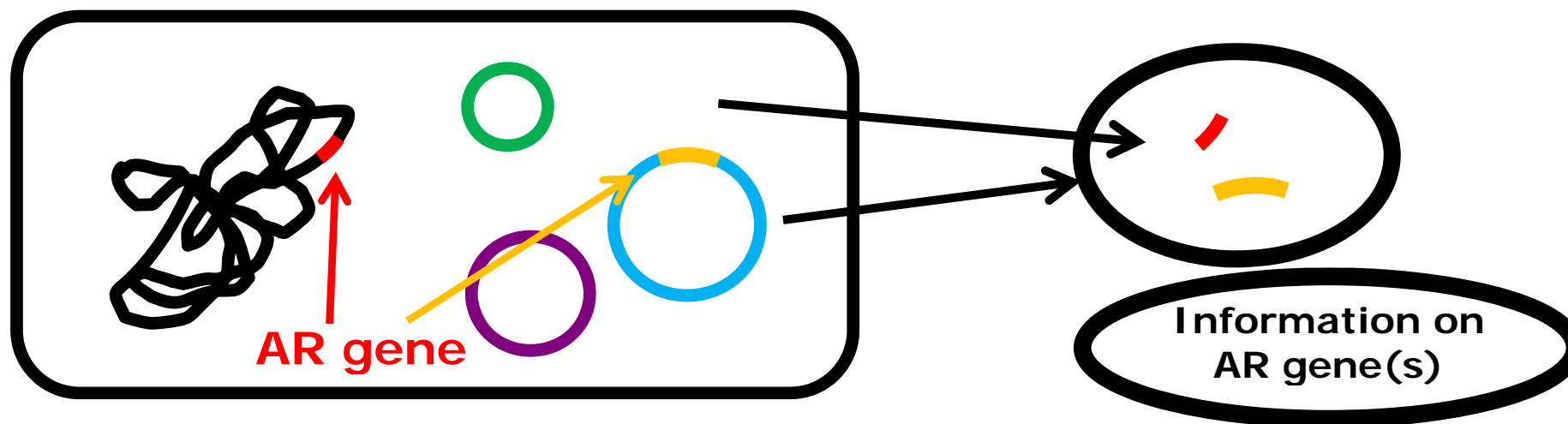
WHAT

WHY

TUESDAY
morning

Singleplex PCR assays
on AR genes of interest
a. beta-lactamase-encod.;
b. PMQR

To confirm microarray results
and to know the exact gene variant
(through sequencing)



...and today:

- Lab theme 2:

1) Read your plates:

Are there suspect MRSA?

2) If yes, use them to perform a multiplex PCR assay for simultaneous detection of *pvl* + *mecA* + *mecA*_{LGA251} + *spa*?



Lab theme 2: MRSA surveillance



WHEN

WHAT

WHY

MONDAY

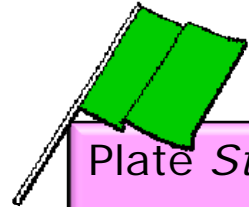


Plate *Staphylococcus aureus* isolates on selective agar plates

To isolate suspect MRSA

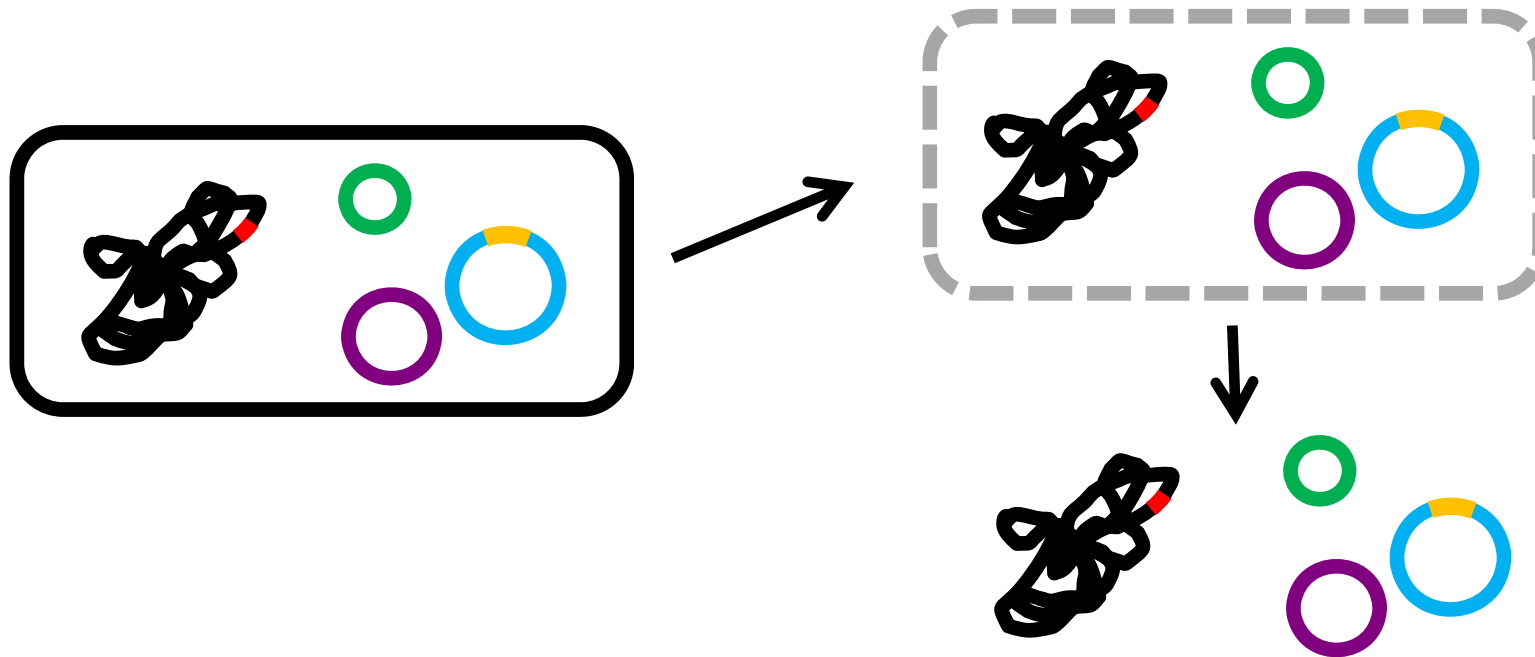
TUESDAY

Multiplex PCR
spa/pvl/mecA/mecA_{LGA251}

To rapidly detect/confirm
(and type) MRSA

PCR

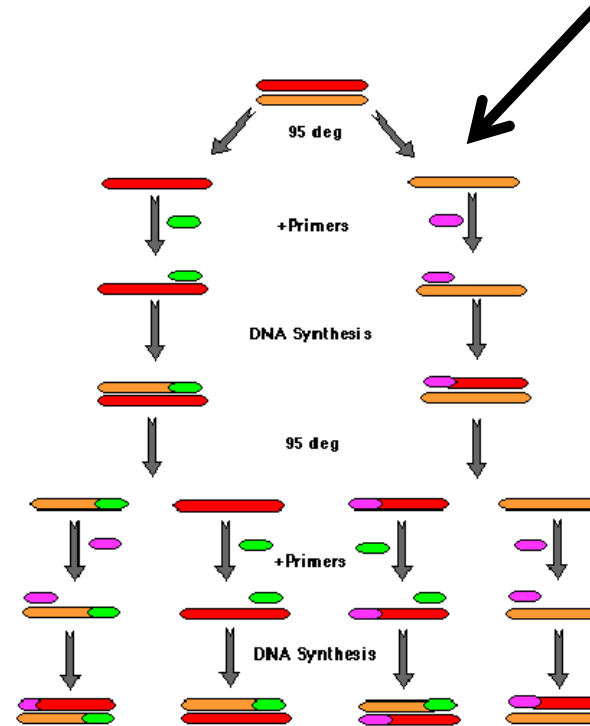
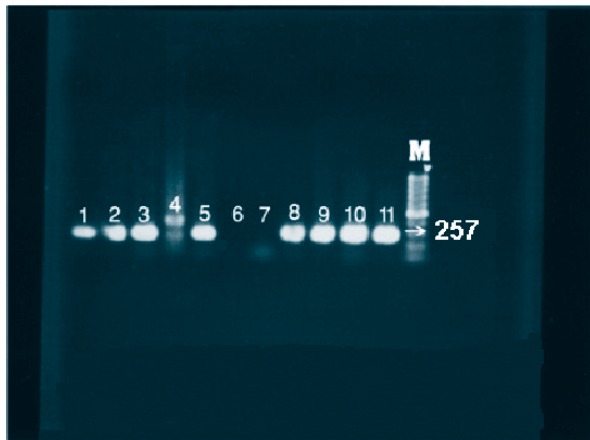
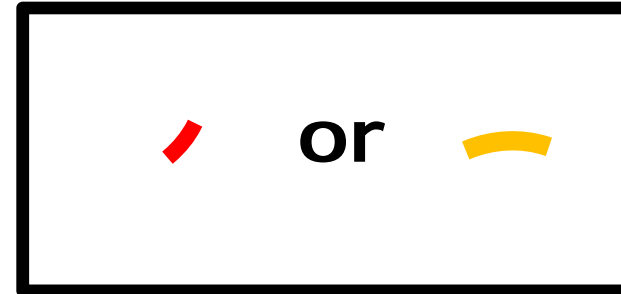
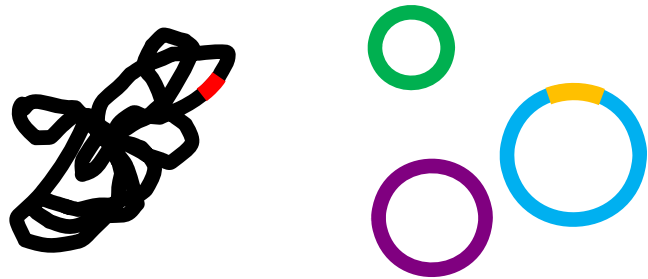
- Before starting the PCR assays, you will prepare DNA lysates (yesterday for microarray: high-quality DNA extracted with a commercial kit)



- Different procedures for Gram-positive and Gram-negative bacteria due to differences in the bacterial cell wall

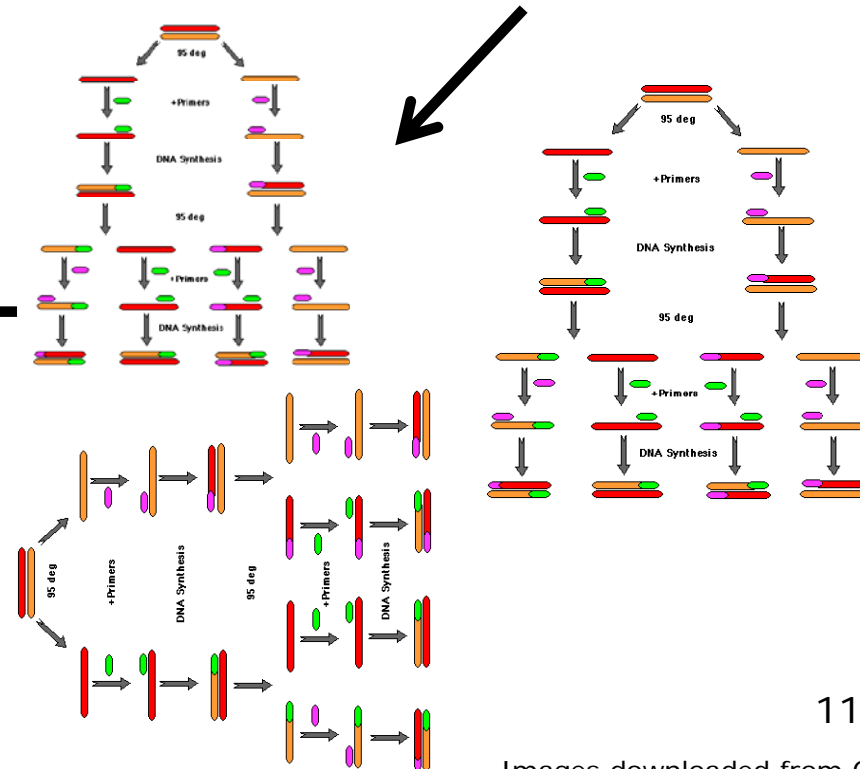
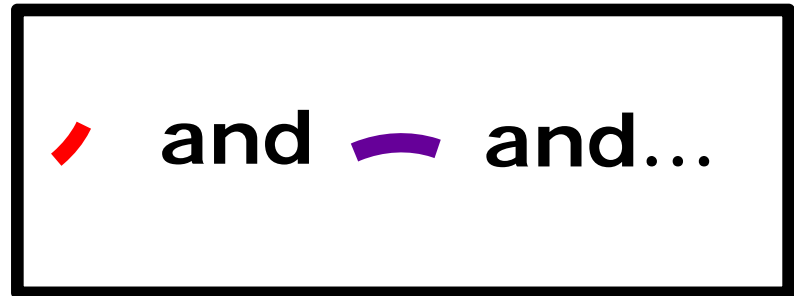
PCR (singleplex)

Your target:



PCR (multiplex)

Your target:



Lab theme 1: Detection and characterization of AR determinants of public health relevance (beta-lactamase-encoding genes & PMQR)



WHEN

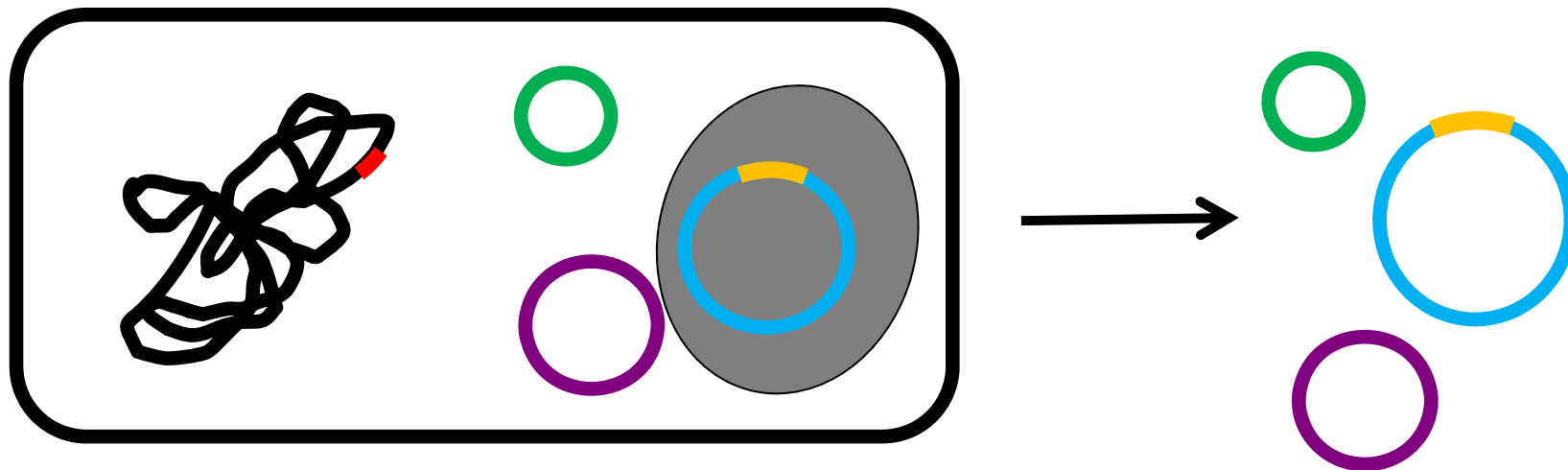
WHAT

WHY

TUESDAY
afternoon

Purification of
plasmid DNA

To isolate the vector of AR genes
of interest for further characterization



Tuesday



Brainstorming

**PCR
PCR
PCR
PCR**

**Henrik
Hasman**

**Eliza
Bielak**

Lunch...

Plasmid extraction

Have a good day!

AUG2	AMOXICILLIN+CLAVULANAT (2:1)
AMP	AMPICILLIN
APR	APRAMYCIN
FOT	CEFOTAXIME
XNL	CEFTIOFUR
CHL	CHLORAMPHENICOL
CIP	CIPROFLOXACIN
COL	COLISTIN
FFN	FLORFENICOL
GEN	GENTAMICIN
NAL	NALIDIXAN
NEO	NEOMYCIN
SPE	SPECTINOMYCIN
STR	STREPTOMYCIN
SMX	SULPHAMETHOXAZOLE
TET	TETRACYCLINE
TMP	TRIMETHOPRIM