

ESCMID summer school 2008, Regensburg, Germany

---

# Current trends in resistance to $\beta$ -lactams and fluoroquinolones in *Enterobacteriaceae*

---

**Hans-Jörg Linde**

Institute for Medical Microbiology and Hygiene

University of Regensburg

*Dictionary.com Unabridged (v 1.1) – Cite This Source – Share This*

**trend**  [Audio Help](#) [\[trend\] Pronunciation Key](#) - [Show IPA Pronunciation](#),

*-noun*

1. the general course or prevailing tendency; drift: *trends in the teaching of foreign languages; the trend of events.*
2. style; vogue: *the new trend in women's apparel.*
3. the general direction followed by a road, river, coastline, or the like.

*-verb (used without object)*

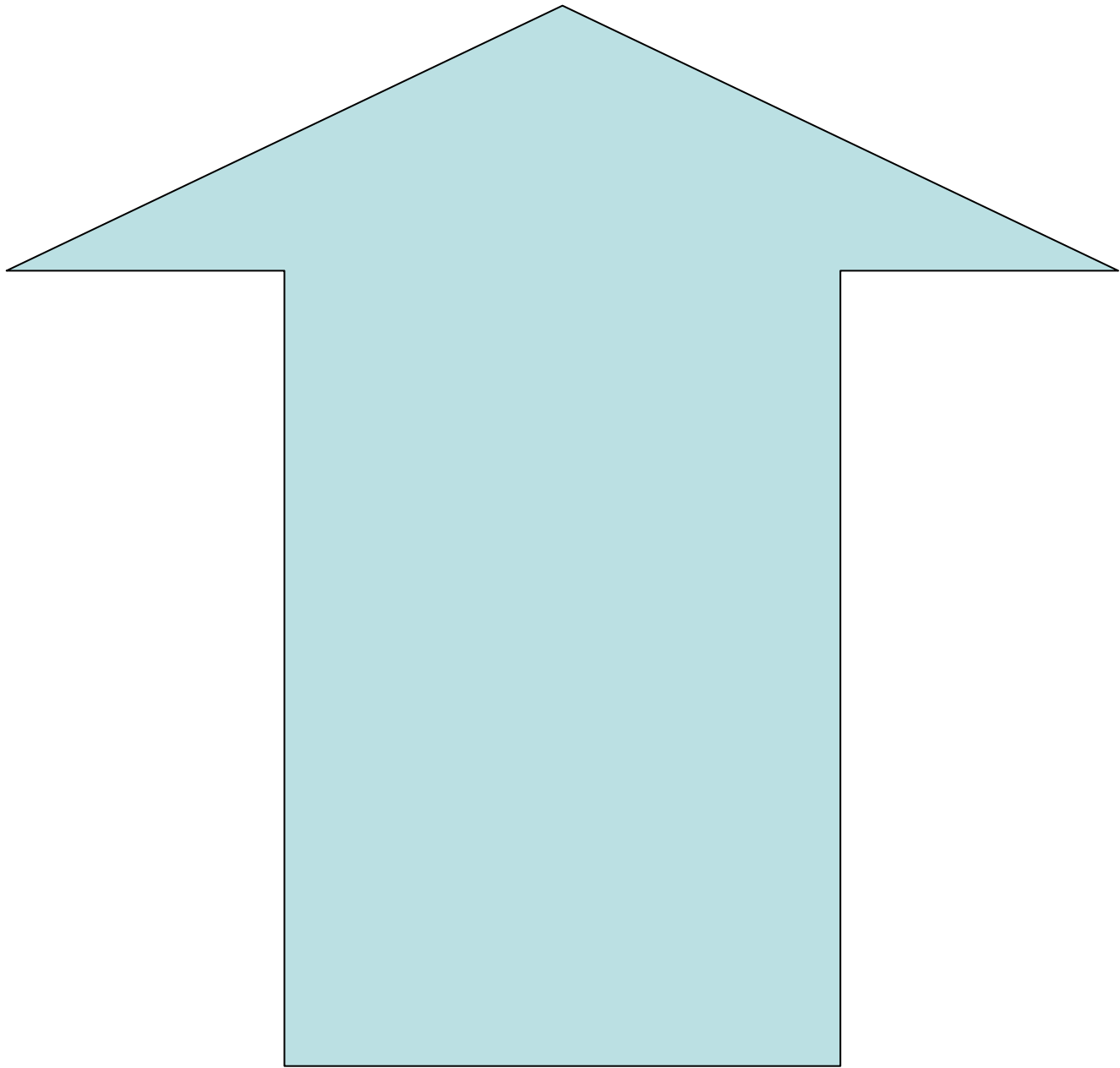
4. to have a general tendency, as events, conditions, etc.
5. to tend to take a particular direction; extend in some direction indicated.
6. to veer or turn off in a specified direction, as a river, mountain range, etc.: *The river trends toward the southeast.*

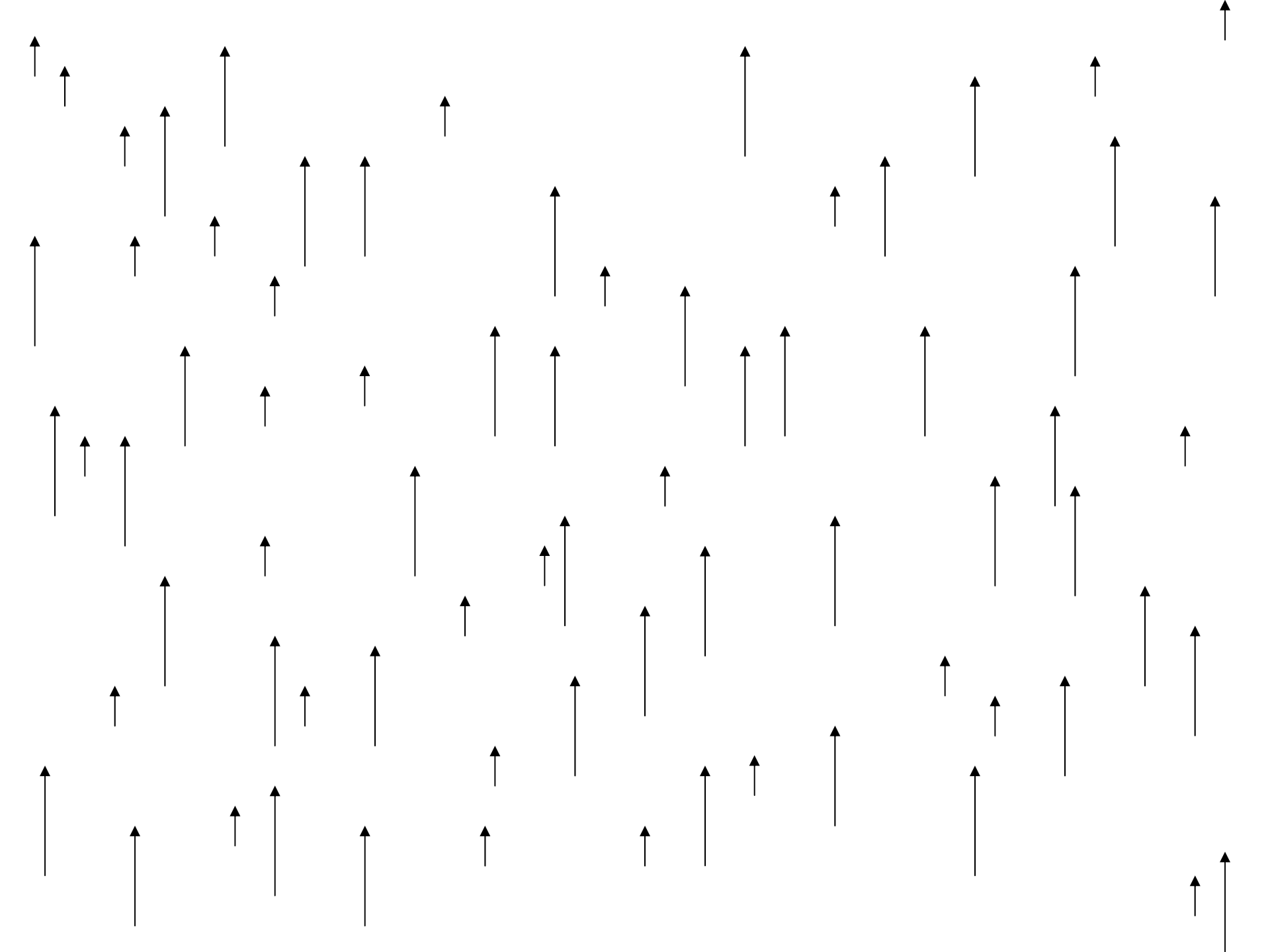
[Origin: bef. 1000; ME *trenden* to turn, roll, OE *trendan*; akin to OE *trinde* ball, D *trent* circumference, Sw *trind* round. See [TRINDLE](#), [TRUNDLE](#) ]

—*Synonyms* 1. See [TENDENCY](#). 5. stretch, run, incline.

*Dictionary.com Unabridged (v 1.1)*

*Based on the Random House Unabridged Dictionary, © Random House, Inc. 2006.*

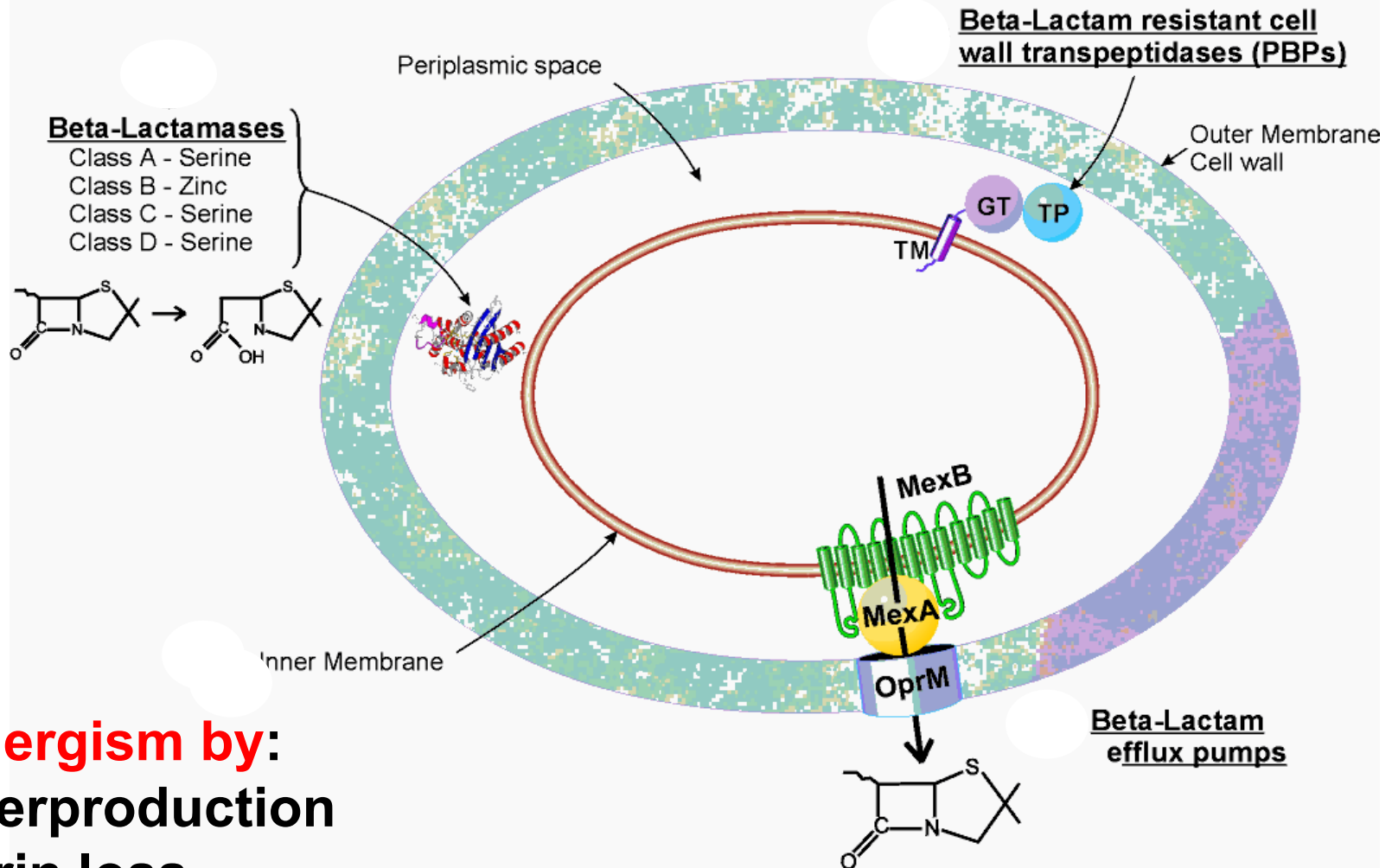




# AGENDA:

- Emerging mechanisms
  - Porins, pumps and enzymes
- *Enterobacteriaceae*
  - well-known members
  - emerging species
  - What is not dealt with (what I would have liked to talk about ...)
- Prevalence of resistance
  - what is happening in different countries

# Resistance mechanisms against $\beta$ -Lactams



## Synergism by:

- Overproduction
- Porin loss
- Efflux

# Clinical sequential isolates - *K. pneumoniae*

Strain	$\beta$ -lactamase			MIC (mg/l)		AAH6''		
	CAZ	CAZ/ CLA	CTX	FOX	IMP	AK	CIP	CLH
LB 1	1	<0.125	0.06	4	0.25	0.5	0.5	>128
LB 2	>256	<0.125	2	4	0.25	32	0.5	>128
LB 3	>256	<0.125	2	4	0.25	32	8	>128
LB 4	>256	8	64	64	0.25	32	8	24

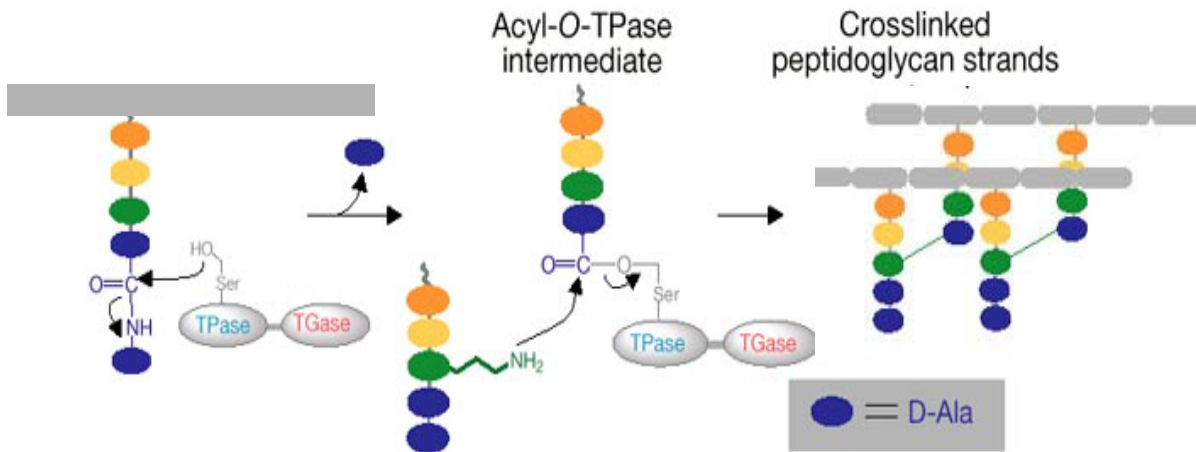
Porin  
Efflux

GyrA

Clonal relationship

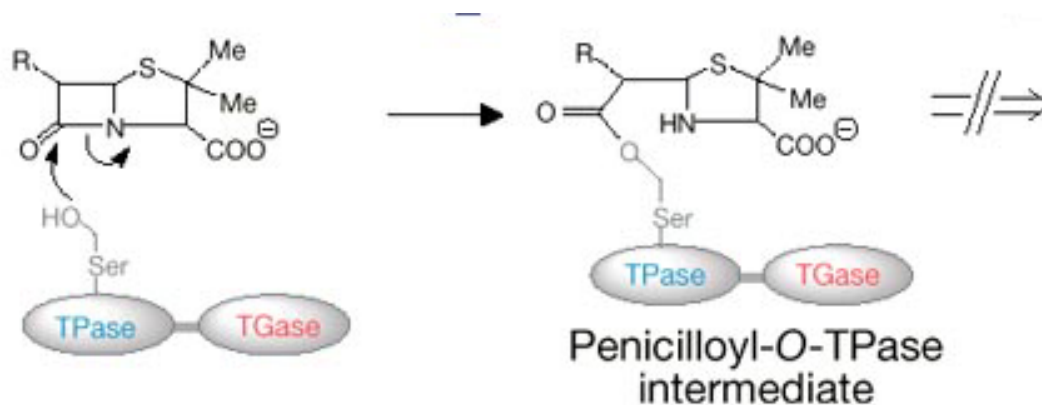
# Die Wirkung von $\beta$ - Lactam Antibiotika

TP ase = Transpeptidase = PBP



Crosslinks

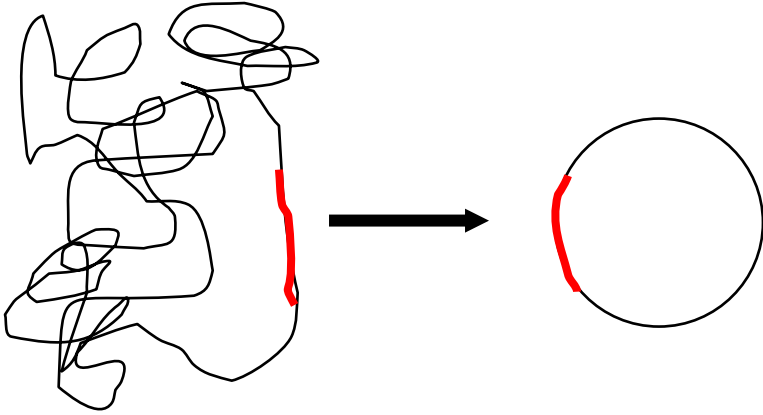
⇒ Bakterien wachsen



Keine Crosslinks

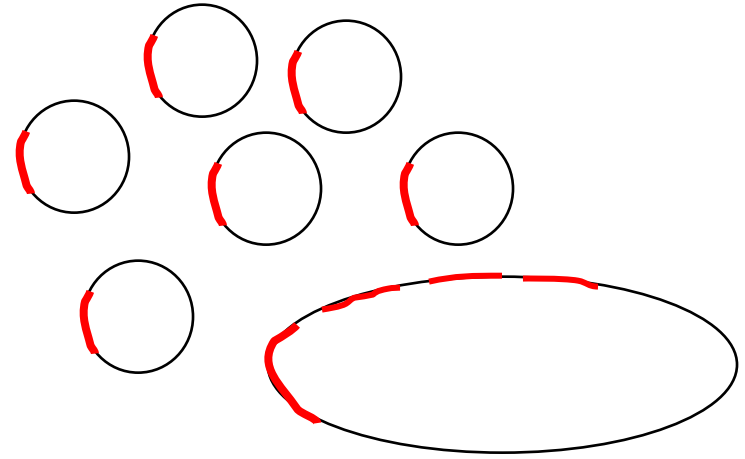
⇒ Bakterien lysieren

## 1. Vom Chromosom auf's Plasmid



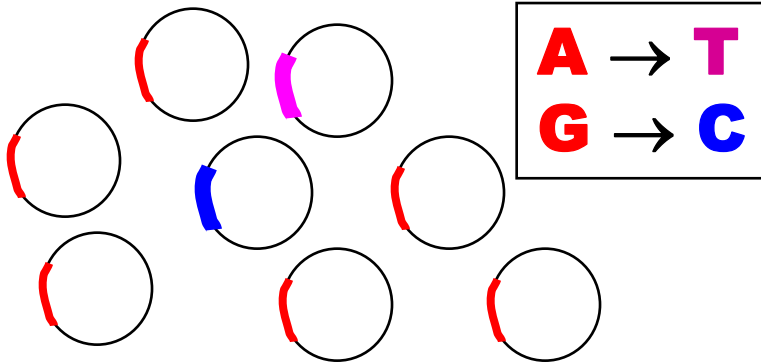
Niedrige Kopiezahl, schlechte Affinität

## 2. Viele Plasmide sind schöner ...



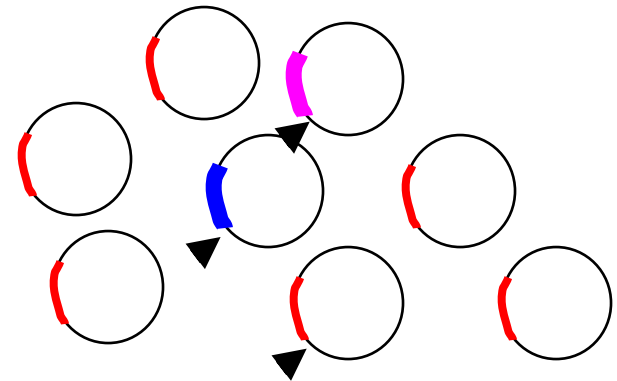
Hohe Kopiezahl, schlechte Affinität

## 3. Verfeinerung des Enzyms



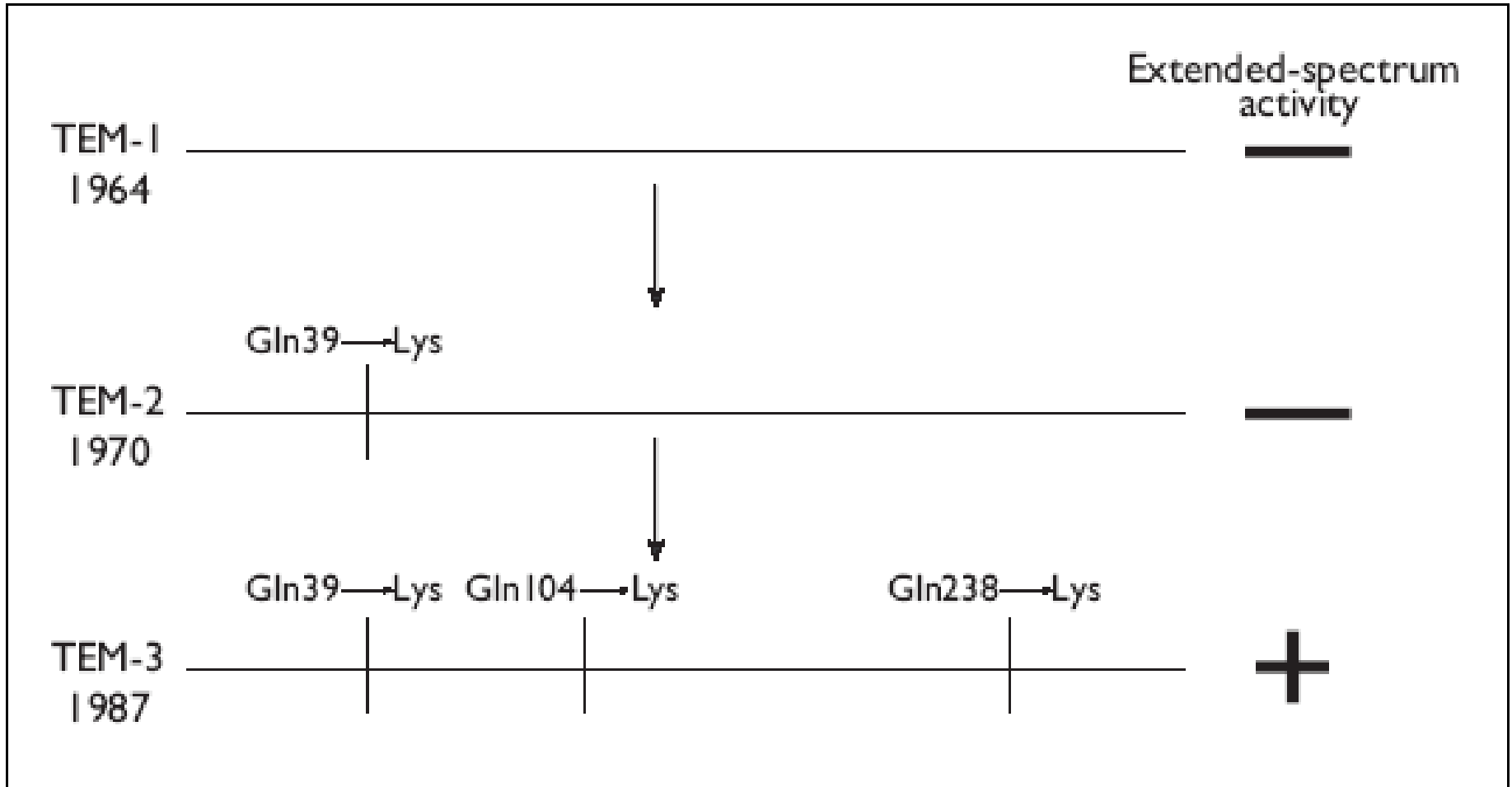
Hohe Kopiezahl, hohe Affinität

## 4. Verfeinerung des Promoters



Hohe Expression, hohe Affinität

# Evolution von TEM-1 zu ESBL



# Rapid evolution of a protein *in vitro* by DNA shuffling

*E. coli* + pTEM-1:  $\text{MHK}_{\text{Cefotaxim}}$

0,02 mg/l → **640 mg/l**

1. PCR
2. DNase-Verdau
3. Rekombinatorisches Assembly
4. Klonierung und Selektion

} „Shuffling“, 3 ×

→ MHK-Veränderung × 32.000

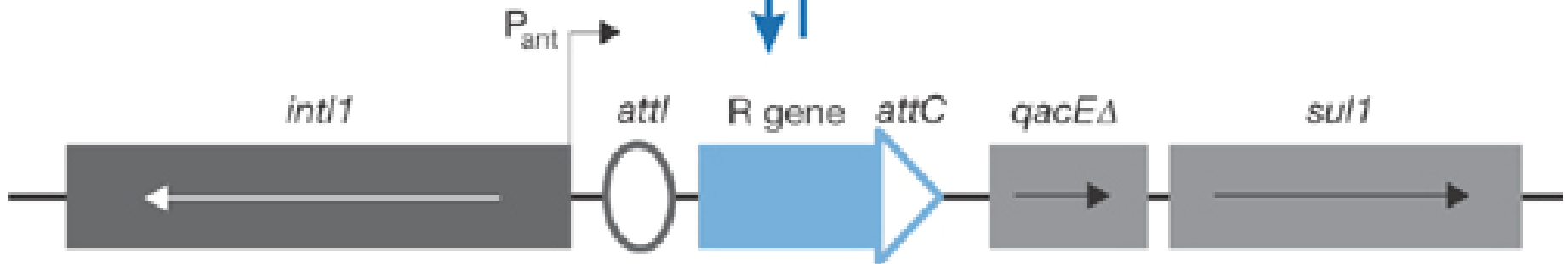
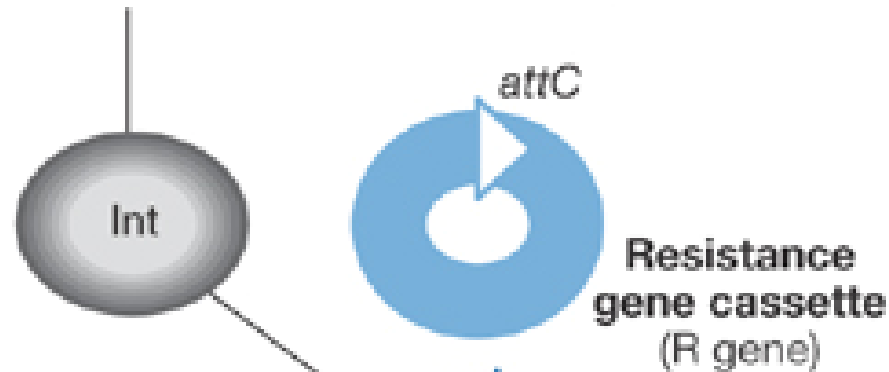
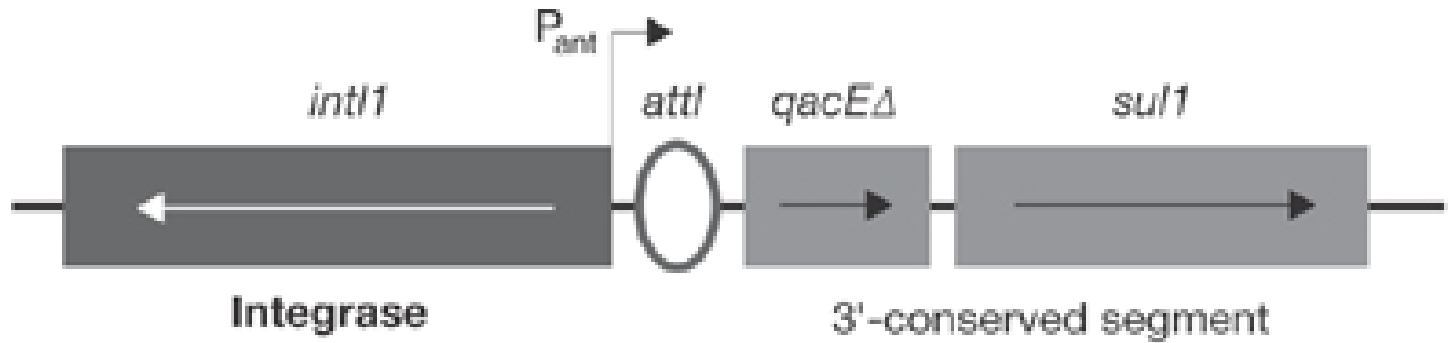
→ Mutationen im Promoter und im Gen (6)

**Auf Bitte der Wiss. Gemeinschaft vernichtet**

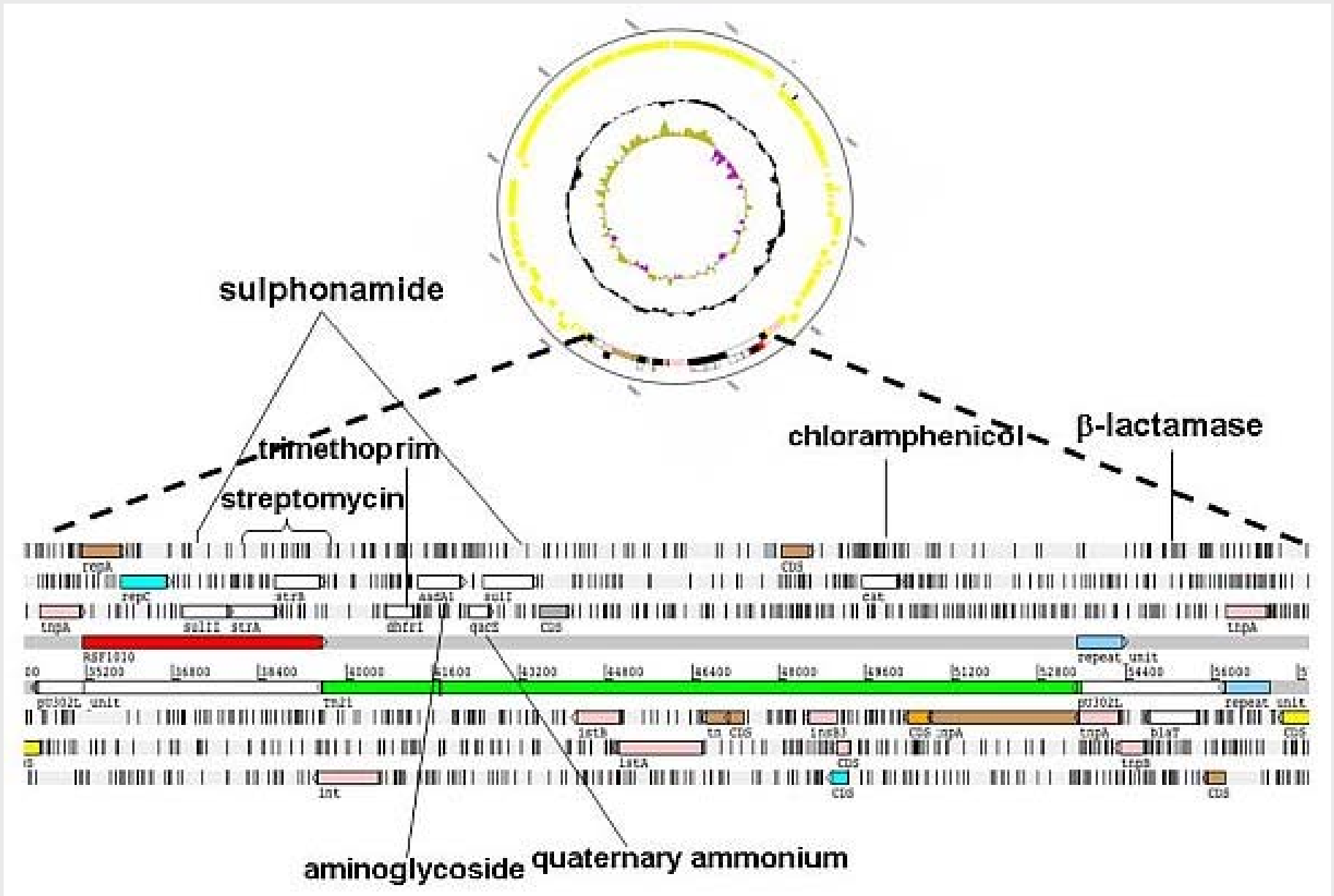
# New $\beta$ -Lactamases ...

Functional group		1995	2000
1	chrom.	32	51
2		136	256
2a	Staph.	20	23
2b	TEM-1	16	16
2be	ESBL	36	119
2br	ESBL-Inh <sup>R</sup>	9	24
3	Metallo- $\beta$ -Lac.	13	24
4	misc.	7	9

„Integrans are mobile DNA elements with the ability to capture genes, notably those encoding antibiotic resistance, by site-specific recombination“



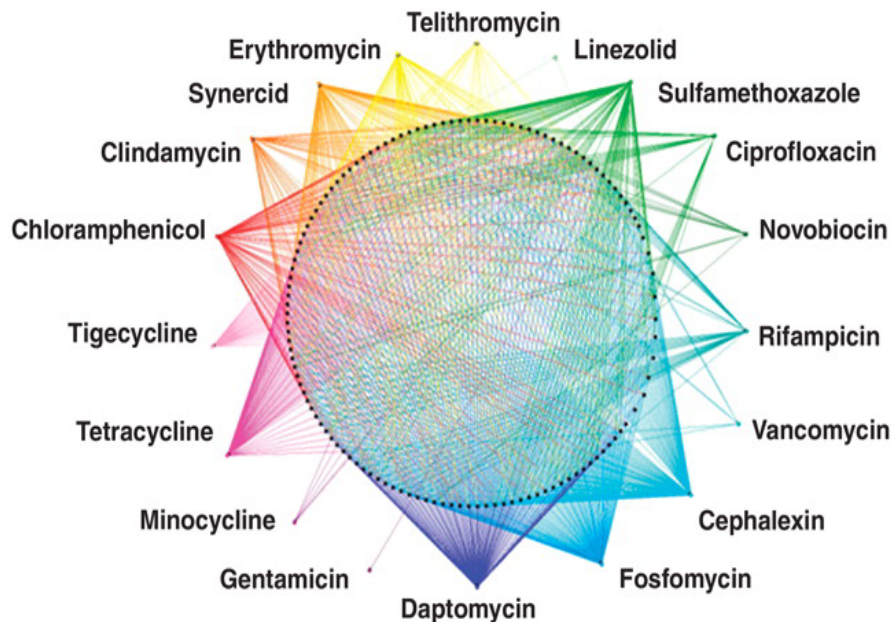
# Virulence plasmid of *Salmonella typhimurium* with antibiotic resistance integron



# Antibiotic Resistome

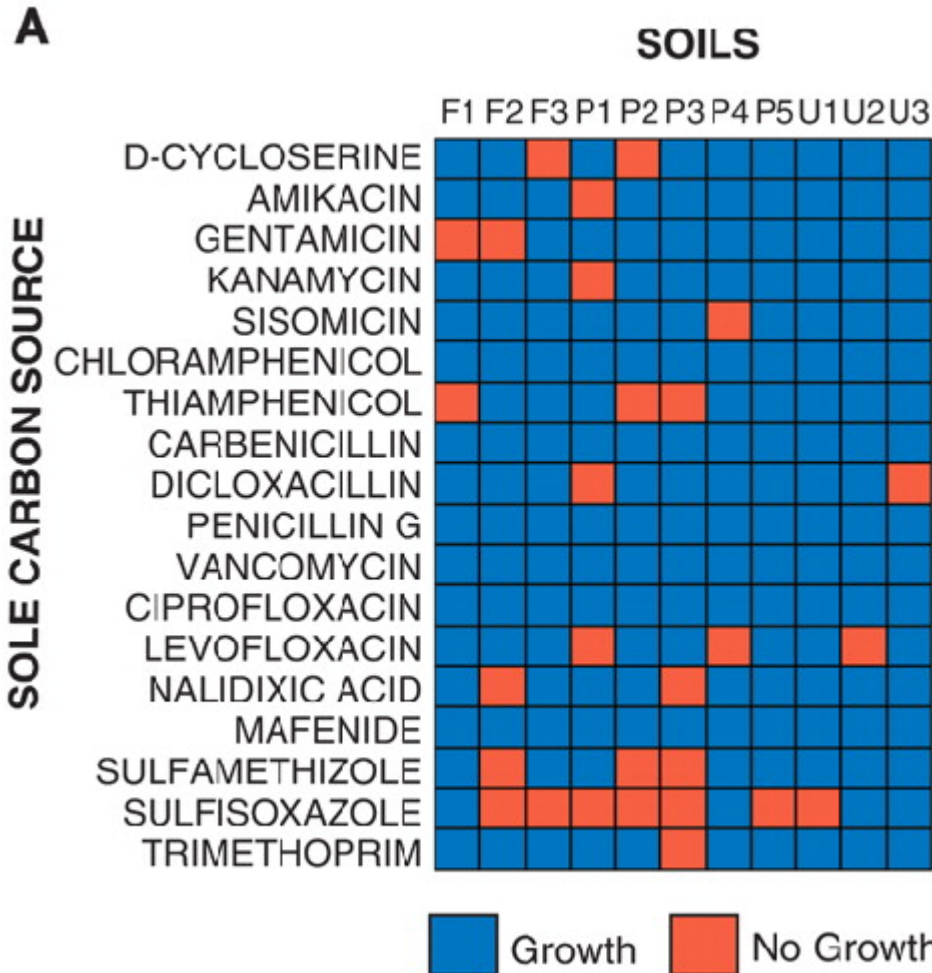
... *Streptomyces* ... synthesize over half of all known antibiotics ...  
 ... resistance elements clustered in antibiotic biosynthetic operons

Screening of 480 soil organisms:



<b>Antibiotic</b>	<b>Resistant (%)</b>
Cephalexin	22
Ciprofloxacin	11
Clindamycin	92
Daptomycin	100
Erythromycin	27
Novobiocin	3
Rifampicin	10
Synercid	61
Telithromycin	17
Trimethoprim	100
Vancomycin	1

# „Soil bacteria are resistant to antimicrobials and a source of resistance determinants“



## Species:

<i>Burkholderiales</i>	41%
<i>Pseudomonadales</i>	24%
<i>Enterobacteriales</i>	13%
<i>Actinomycetales</i>	7%
<i>Rhizobiales</i>	7%
<i>Sphingobacteriales</i>	6%

„*Burkholderia*, *Serratia*“ (!)

# Erreger mit ESBL

---

*Klebsiella pneumoniae*

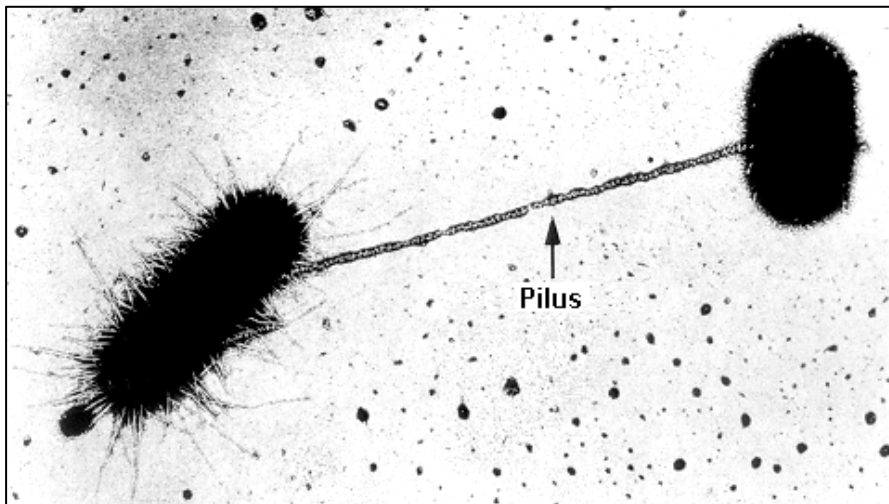
*Escherichia coli*

*Klebsiella oxytoca*

*Proteus mirabilis*

*Salmonella enterica*

*Citrobacter diversus*



*Enterobacter spp.*

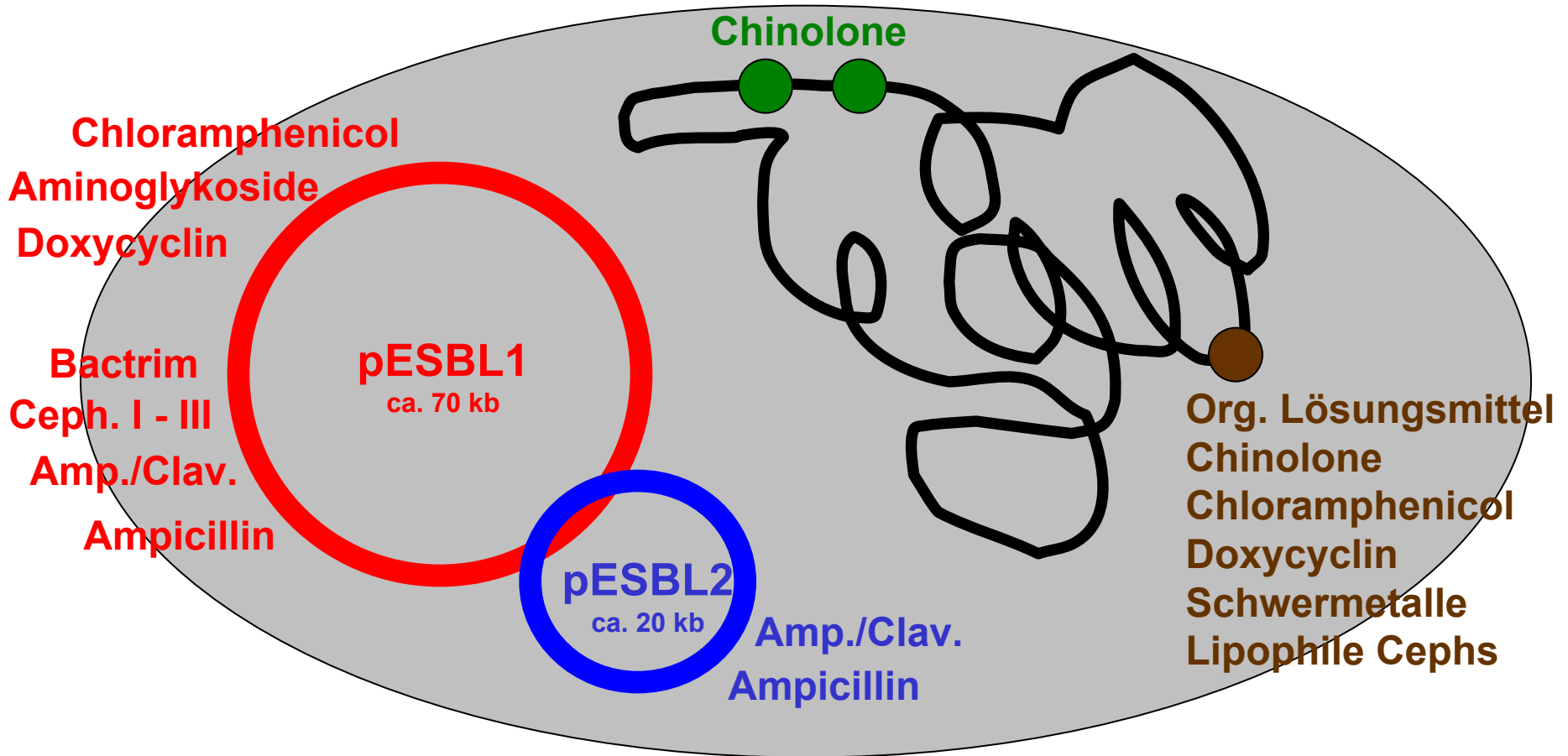
*Citrobacter spp.*

*Morganella morganii*

*Pseudomonas aeruginosa*

# Multiresistenz am Beispiel von *Klebsiella pneumoniae* „ESBL“

= „extended spectrum  $\beta$ -lactamase“



# Fluorquinolones: Resistance mechanisms

## Target sensitivity/protection

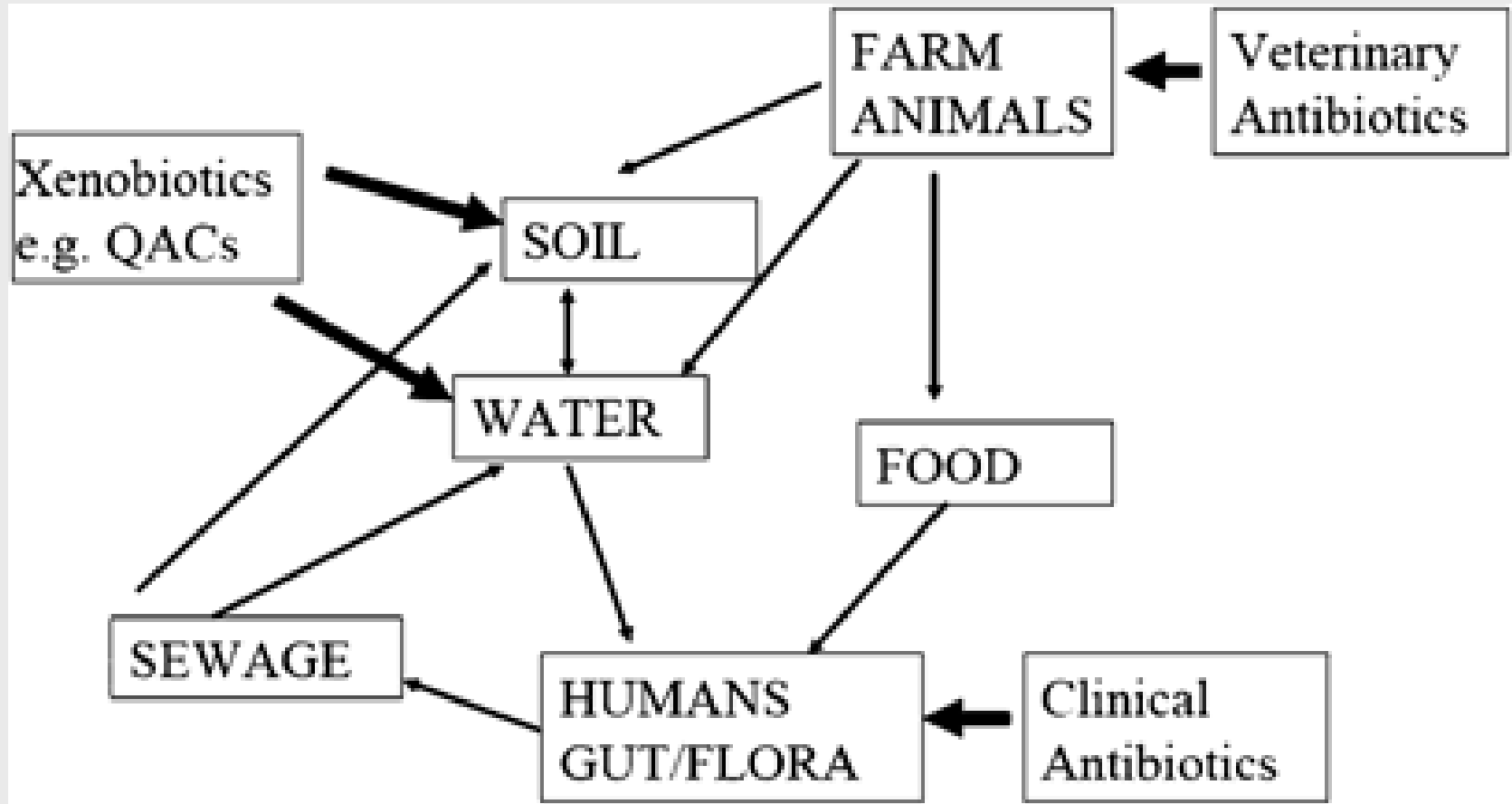
- **Topoisomerase: Point mutations in QRDR**  
GyrA, parC
- **Topoisomerase: 4<sup>th</sup> binding partner**
- QnrA-D plasmid-borne

## Uptake

- **Efflux: Overexpression**  
AcrAB-TolC, AcrEF, AcrD, MdfA, MdtH, MdtK, MdtM; QepA plasmid-borne
- **Porin: Downregulation**  
OmpF

## Inactivation

- **FQ: Inactivation – plasmid borne**  
aac(6')Ib



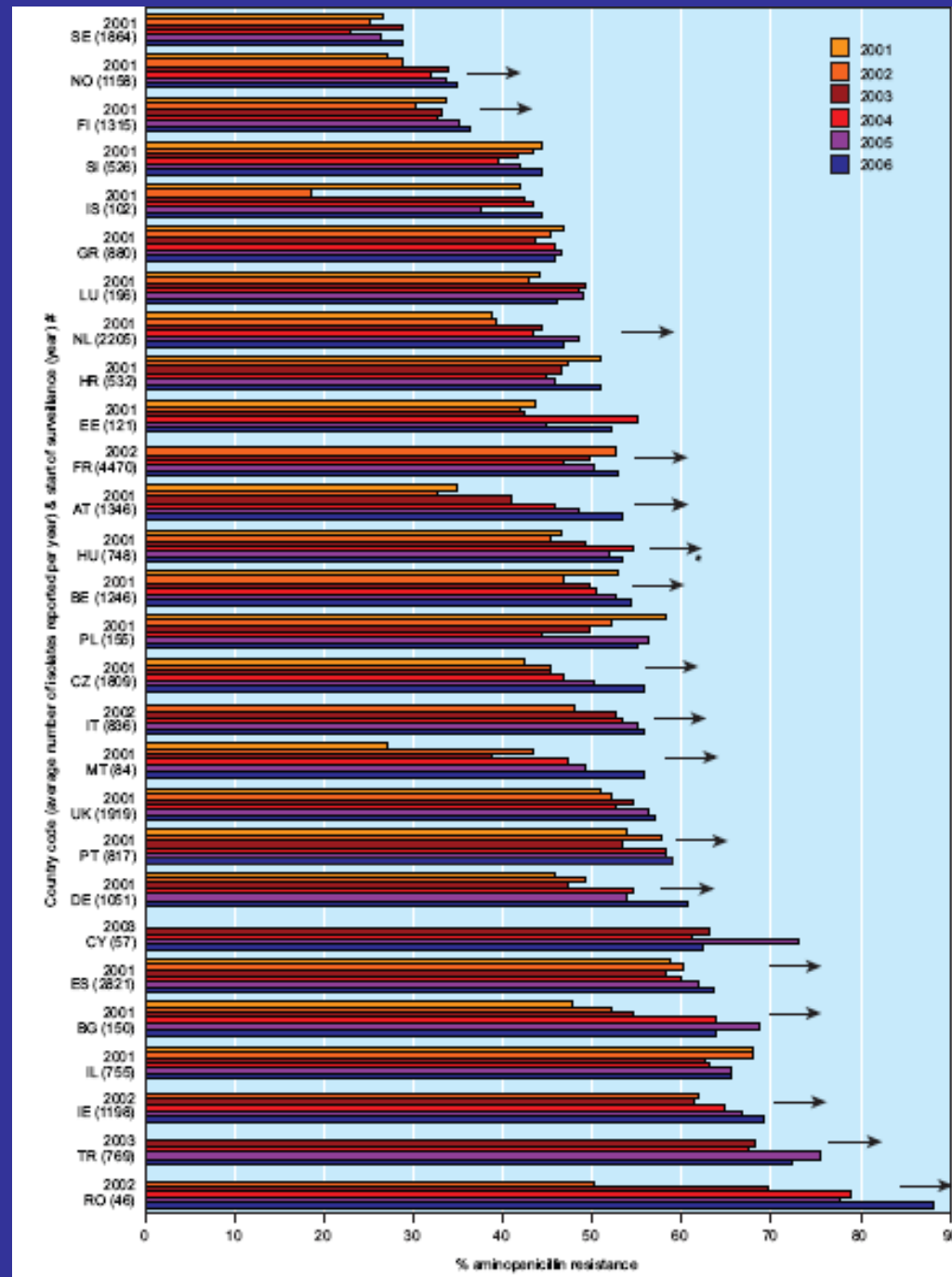
Flow of antibiotic resistance genes in *E. coli* in the biosphere.



# EARSS resistance

## Aminopenicillin *Escherichia coli* 2001–2006

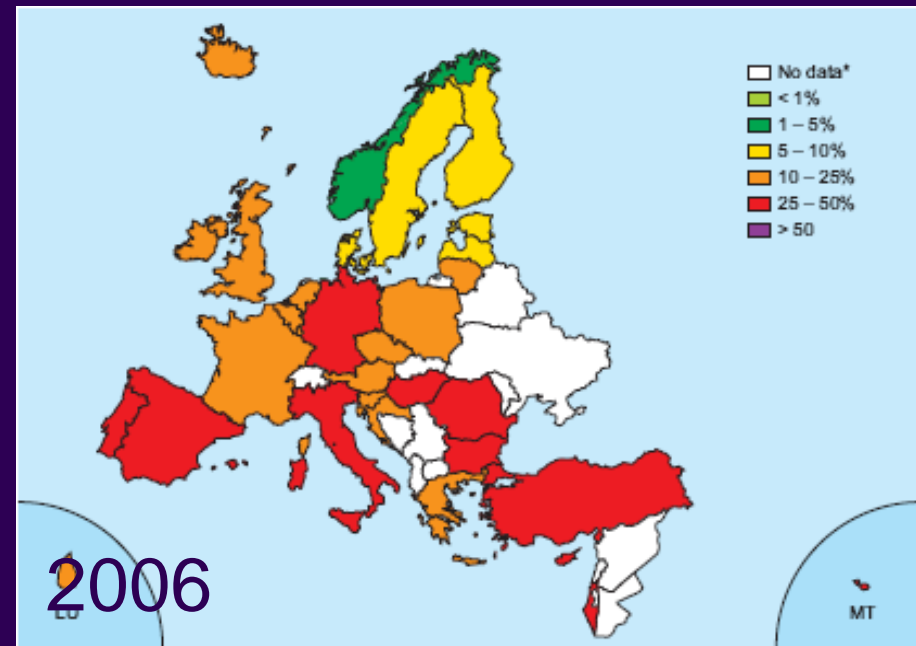
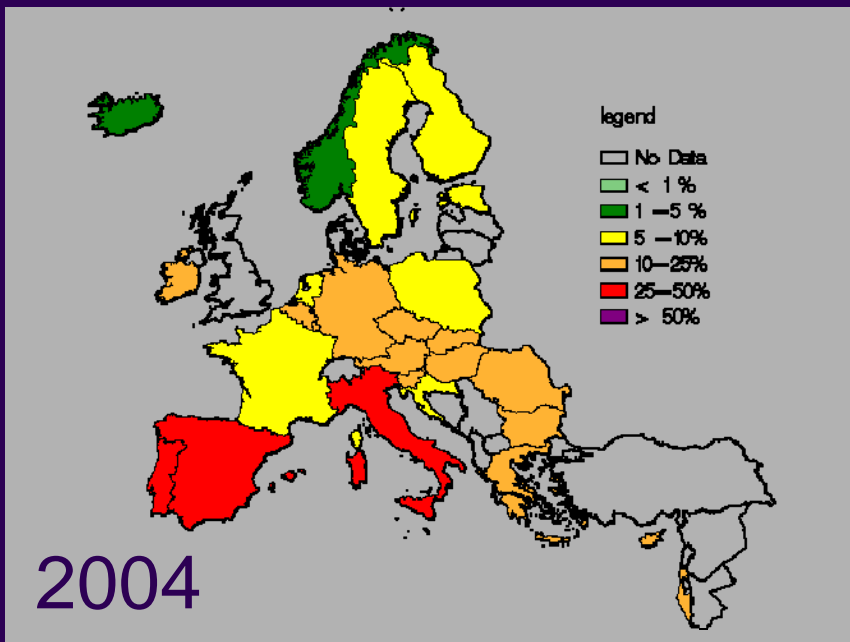
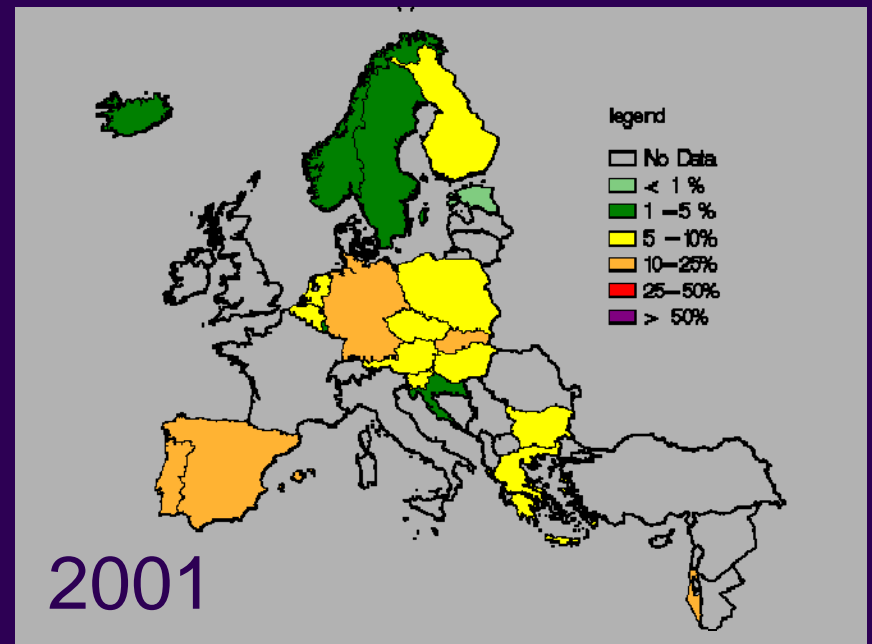
<http://www.earss.rivm.nl>



# EARSS resistance

## Fluoro-quinolones *Escherichia coli*

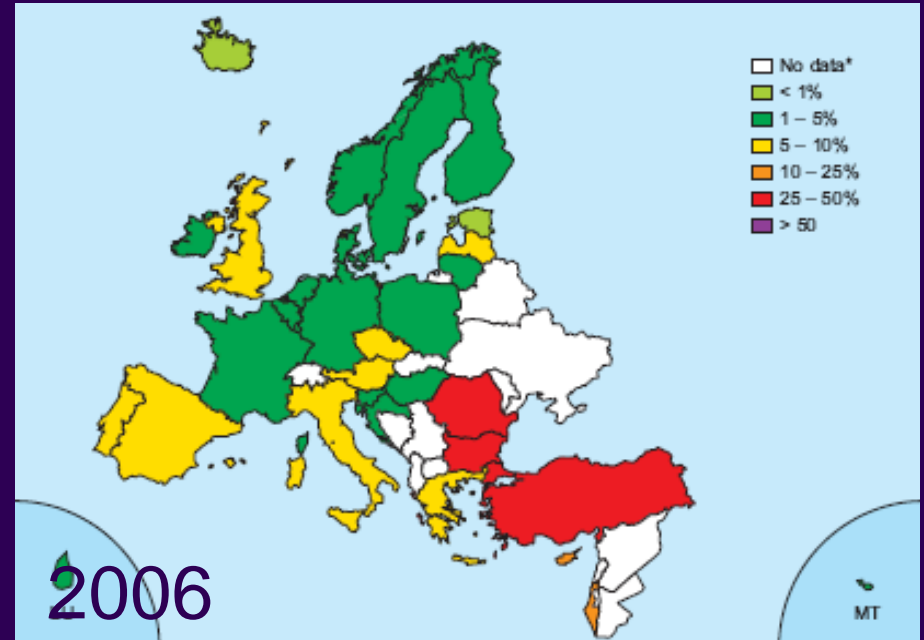
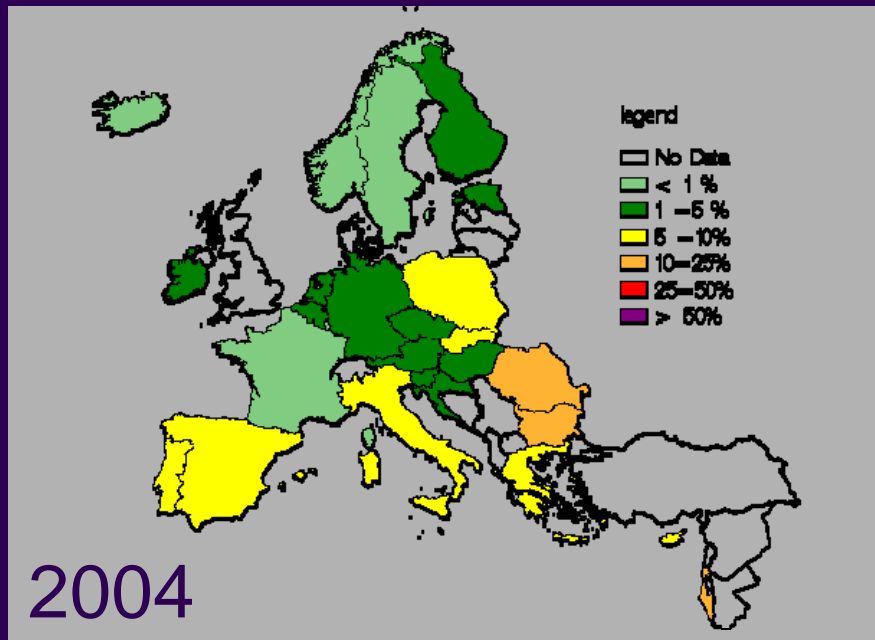
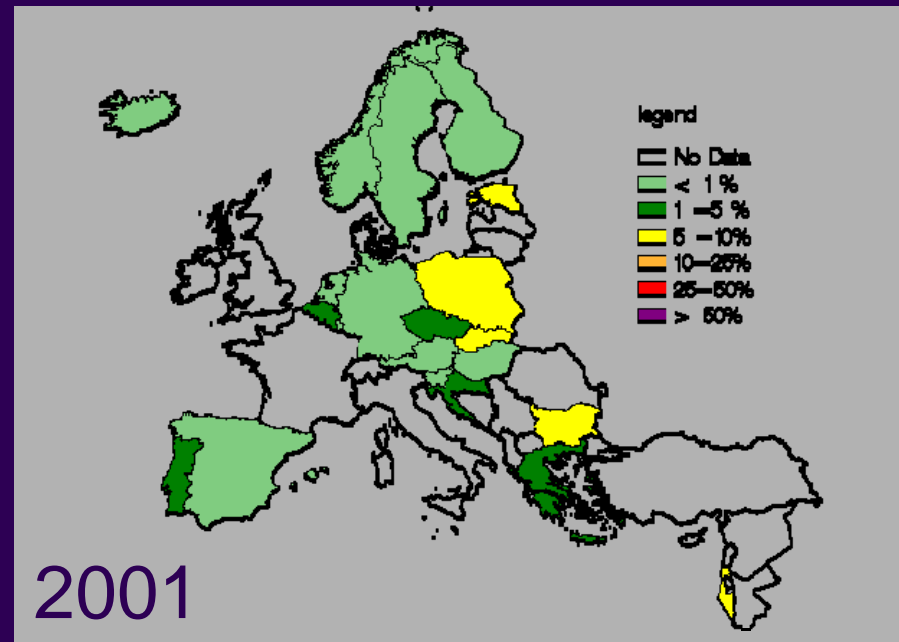
<http://www.earss.rivm.nl>



# EARSS resistance

## 3-gen cephs *Escherichia coli*

<http://www.earss.rivm.nl>



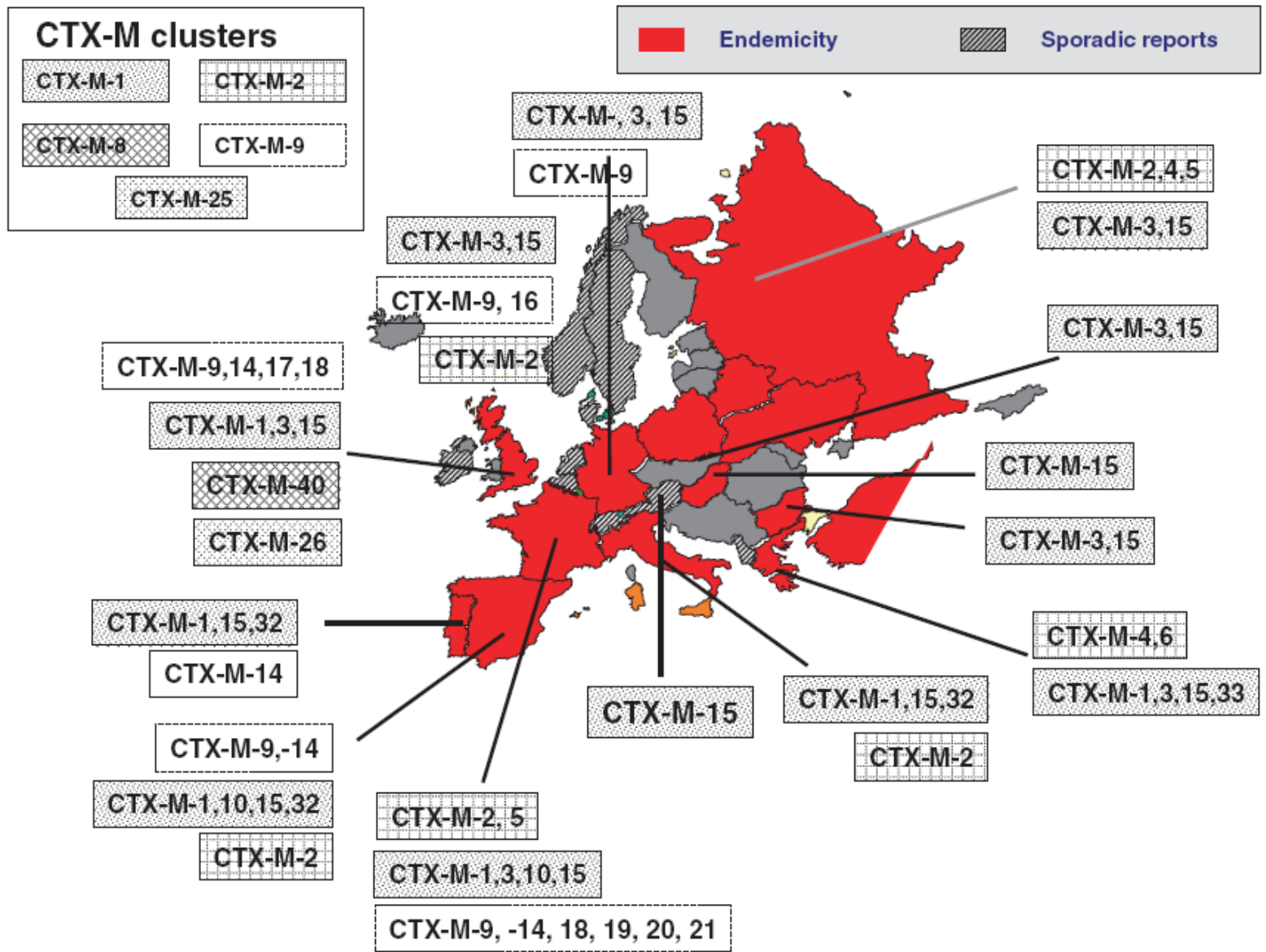
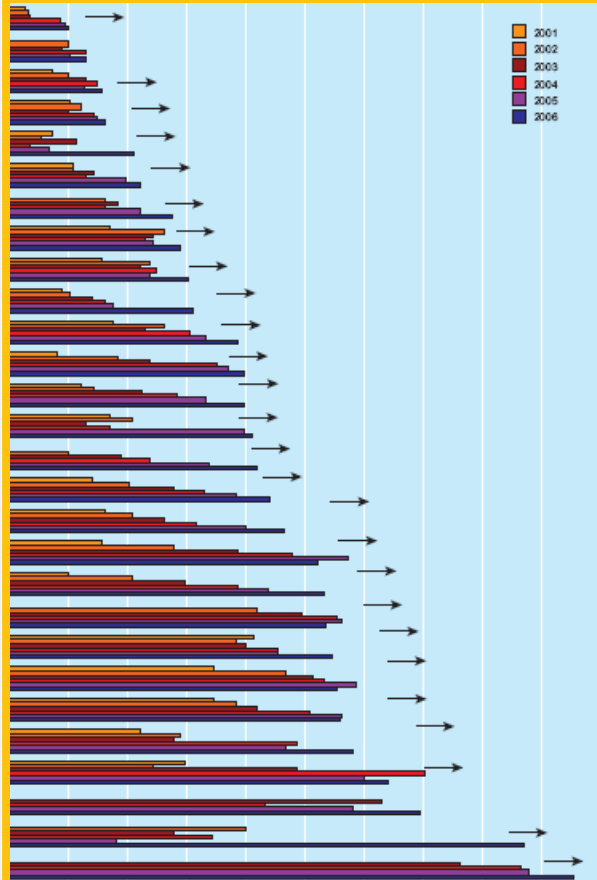


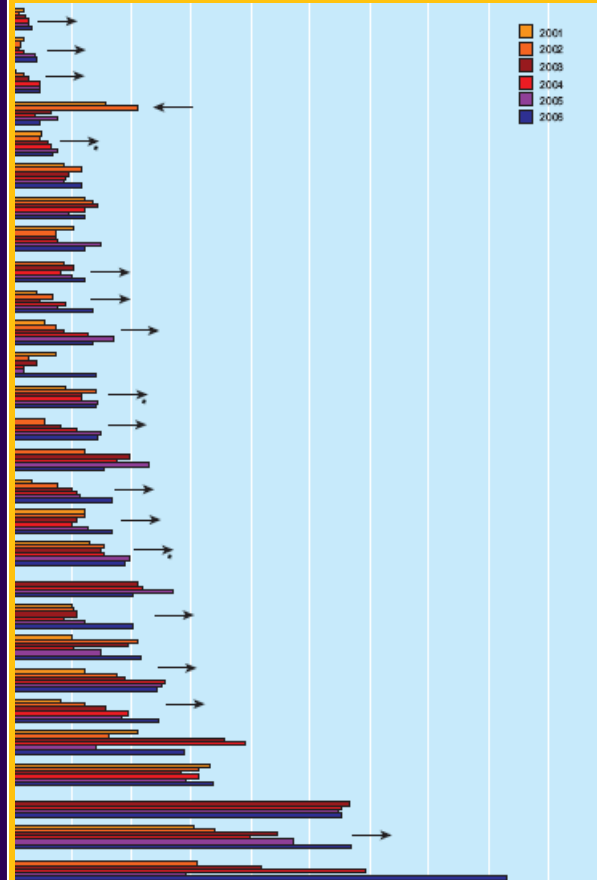
Fig. 1. Distribution of CTX-M enzymes in Europe.

# EARSS resistance: *Escherichia coli*, 2006

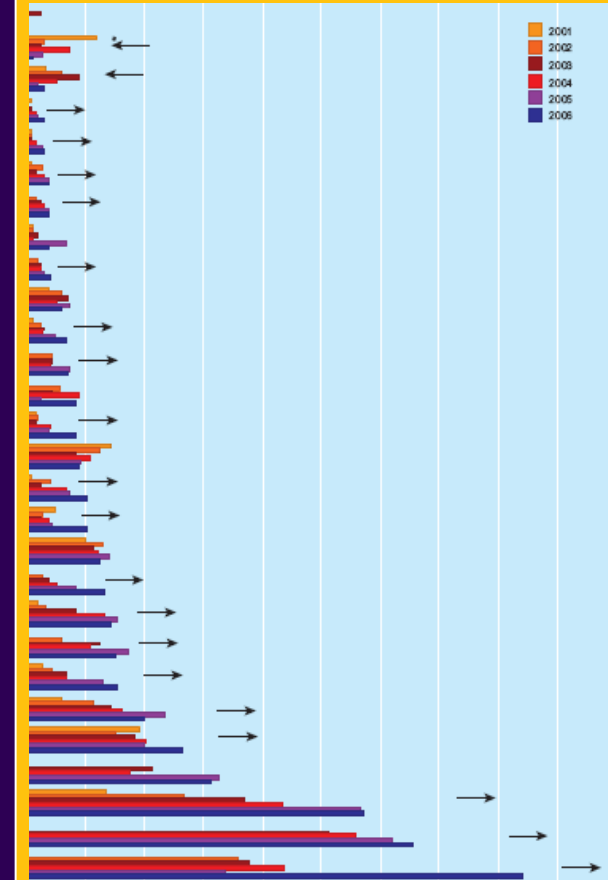
<http://www.earss.rivm.nl>



FQ



AG

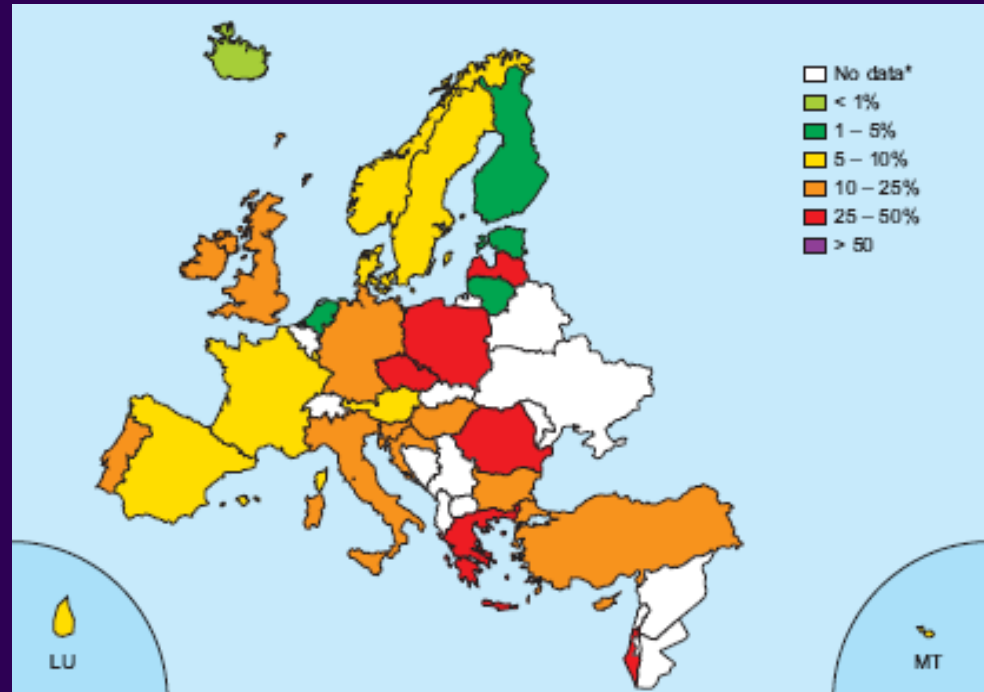


3-gen ceph

# EARSS resistance

Fluoro-quinolones  
*Klebsiella* spp.

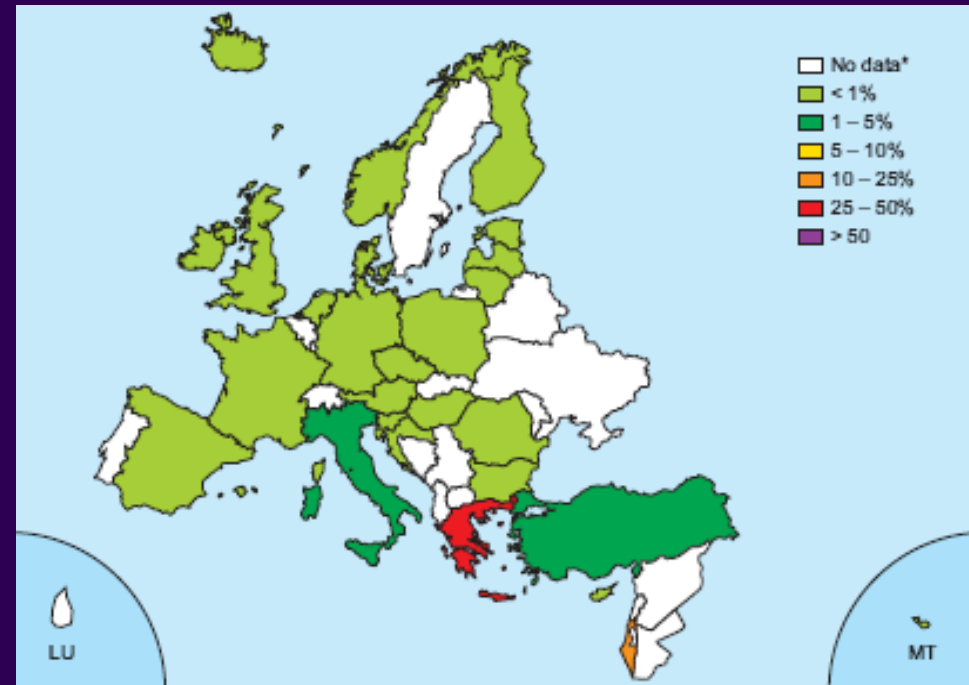
<http://www.earss.rivm.nl>



# EARSS resistance

Carbapenems  
*Klebsiella* spp.

<http://www.earss.rivm.nl>



# EARSS resistance

**FQ + 3-gen + AG**  
***Klebsiella* spp.**

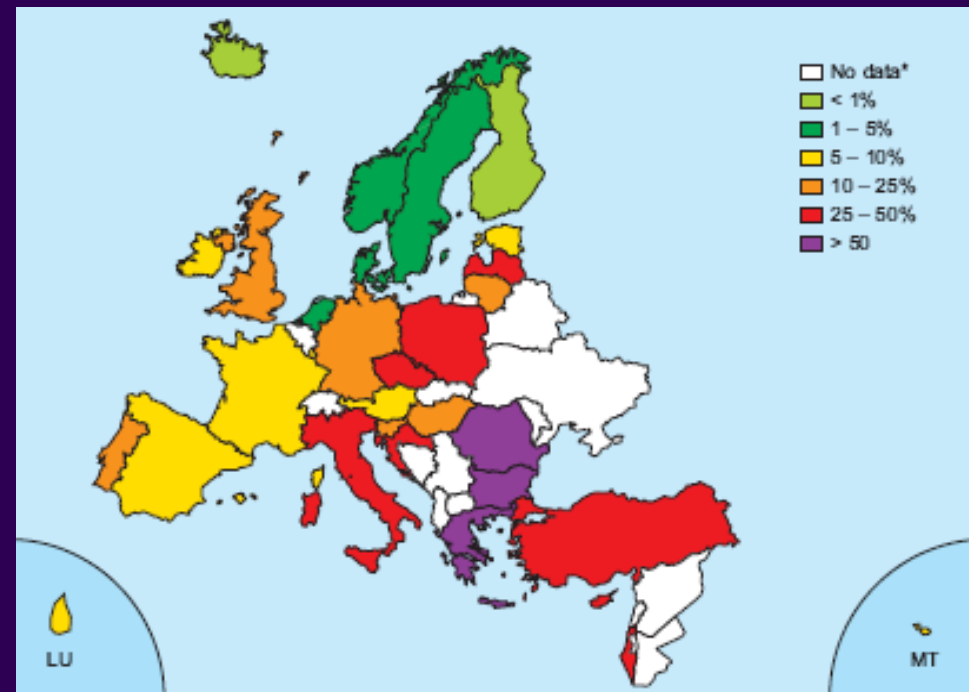
<http://www.earss.rivm.nl>

Resistance pattern	Number	% of total
Full susceptibility*	6,130	70.1%
Single resistance (to indicated drug classes)		
Fluoroquinolones	358	4.1%
3 <sup>rd</sup> generation cephalosporins	217	2.5%
Aminoglycosides	134	1.5%
Resistance to two classes of antimicrobial drugs		
Fluoroquinolones + 3 <sup>rd</sup> generation cephalosporins	281	3.2%
Fluoroquinolones + aminoglycosides	198	2.3%
3 <sup>rd</sup> generation cephalosporins + aminoglycosides	406	4.6%
Resistance to three classes of antimicrobial drugs		
Fluoroquinolones + 3 <sup>rd</sup> generation cephalosporins + aminoglycosides	1,027	11.7%

# EARSS resistance

3-gen cephs  
*Klebsiella* spp.

<http://www.earss.rivm.nl>



# ESBL-related infections

## Community onset

*Escherichia coli*

CTX-M <sup>(15)</sup>

UTI; bacteraemia,  
gastroenteritis

Pens, Cefs,  
+ FQ, SXT, AG

Polyclonal > clusters

UTI, AB, nursing home,  
diabetes

Europe, South America

## Hospital onset

*Klebsiella* spp., others

SHV <sup>(2, 5)</sup>, TEM <sup>(26, 51)</sup>

Nosocomial infections

Pens, Cefs,  
+ FQ, SXT, AG

Polyclonal, clusters

Hospital, chronic disease,  
AB, device

Worldwide

**„Carriage of ESBL<sup>+</sup> Enterobacteriaceae in the lower gastrointestinal tract is an important factor in the subsequent development of clinical infection“**

- Spain

	Hospital	Community
1991	0.3%	0.7%
2003	11.8%	5.5%
- China + India

2006	estimated 10% in 2.5 billion ~ $2.5 \times 10^8$ human carriers	
------	--------------------------------------------------------------------	--

# „Collateral damage“ by carbapenem use

---

- **Carbapenem resistance in EB**  
Porin loss, evolution and spread of carbapenemases
- **Carbapenem resistance in others**  
*e.g. Pseudomonas, Acinetobacter*
- **Superinfektions**  
MRSA, VRE, *Stenotrophomonas*

# Carbapenemases in *Klebsiella*

- ESBL + porin loss
- KPC1 / 2 / 3 (Bush group 2f)
- 2001: New York → USA; South America, China, Israel
- Conjugative plasmid: *Klebsiella*, *C. freundii*, *S. marcescens*, *E. coli*, *Enterobacter*, *Salmonella*, *P. aeruginosa*
- Europe: France, Greece
- 2008: Germany: *Klebsiella pneumoniae*: Cef3, FQ, AG, SXT; Ertapenem!, most isolates resistant to tigecyclin

WER HÄTTE GEDACHT,  
DASS ONKEL HERBERT  
NICHT RESISTENT IST!

