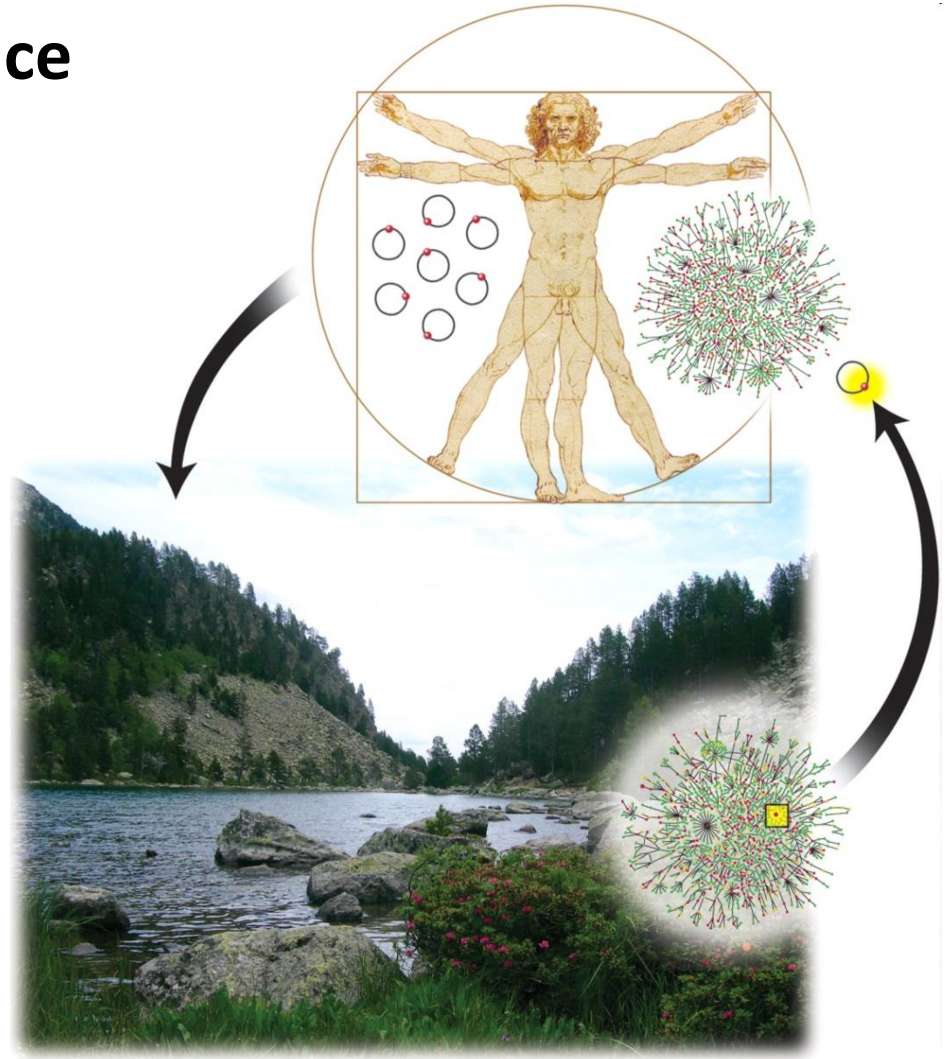
Ecology of Antibiotic Resistance

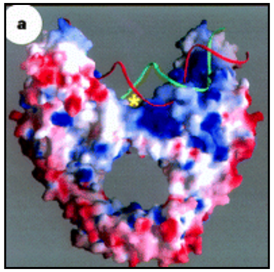
Wolfgang Witte, RKI-Fellow



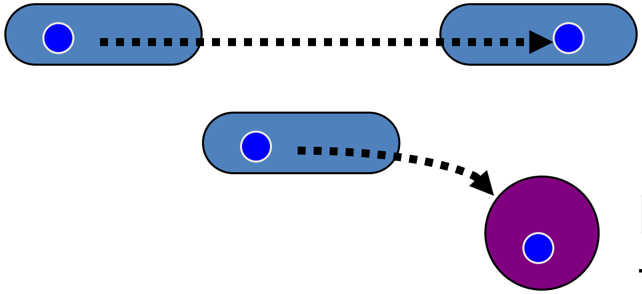
(J. Martinez, Science 2008; 231: 366-367).

Genetic events

- Mutations; e.g. alteration of targets



- Horizontal tranmsfer of resistance genes



homologousT transfer

heterologous transfer

Selective pressure :

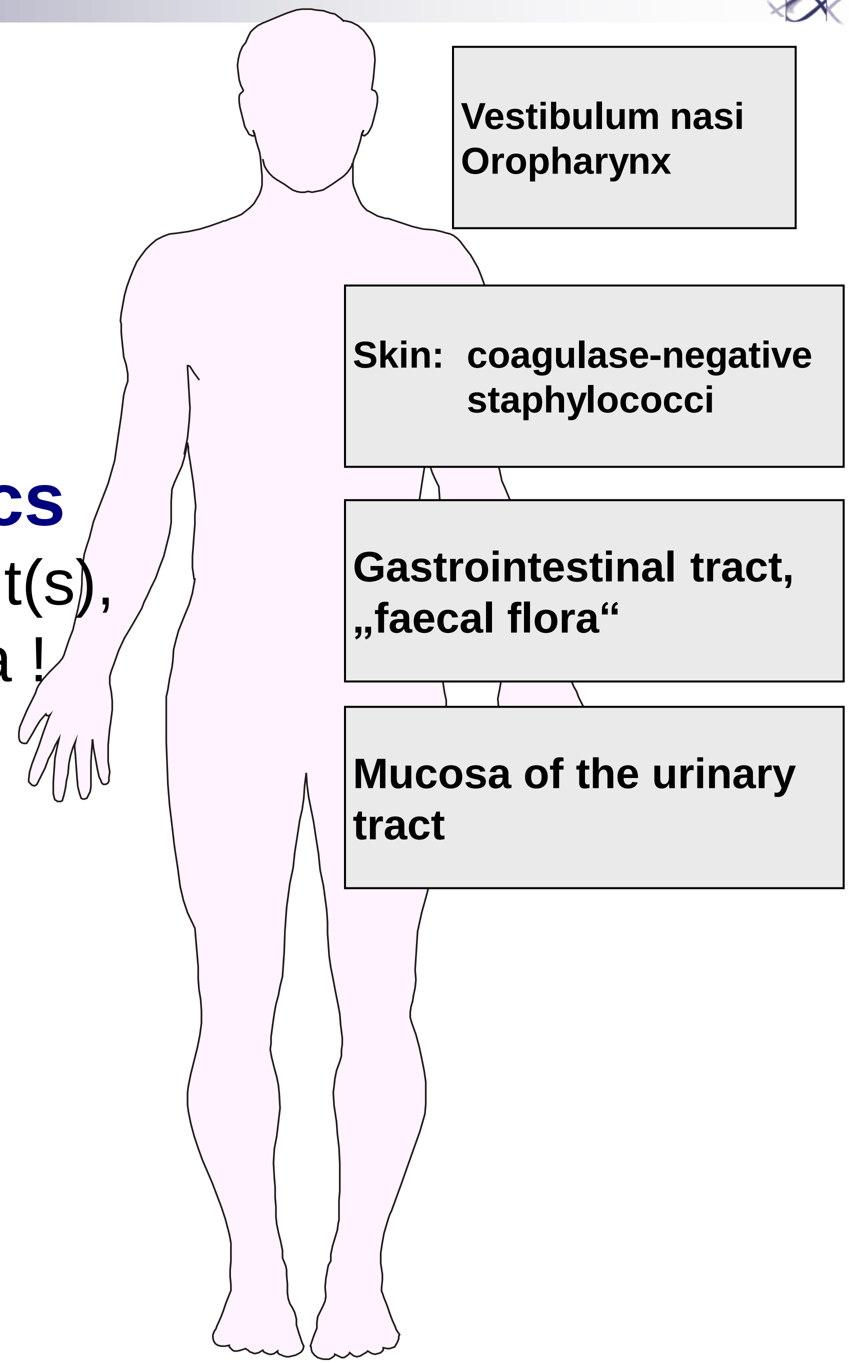
Antibiotics in soil (antibiotii aproducers); usage in human- und veterinary medicicin

„Newcomers “ as colonizer

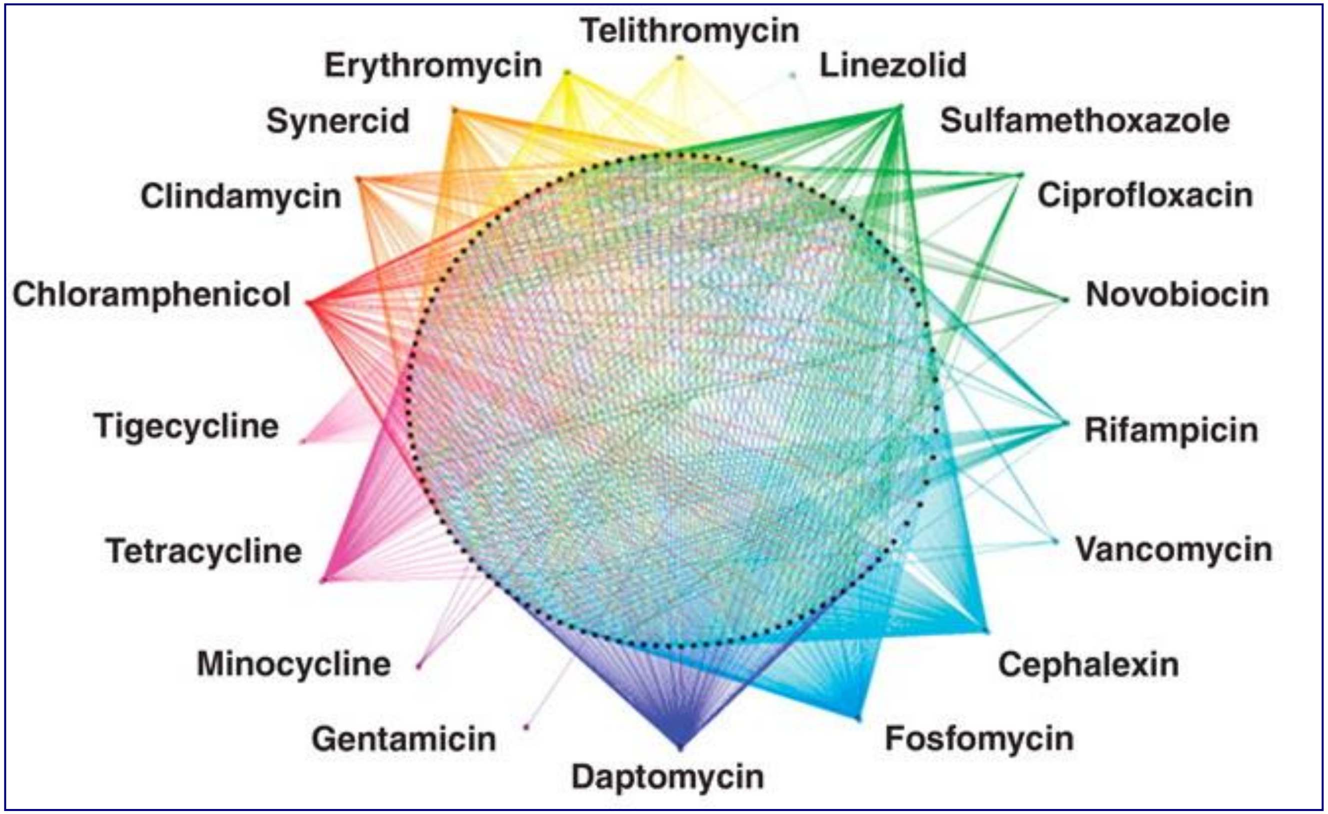
Natural epitopes und microbiota,
Competing microflora

Selective pressure, antibiotics

Not only against the infectious agent(s),
always against colonizing microflora !



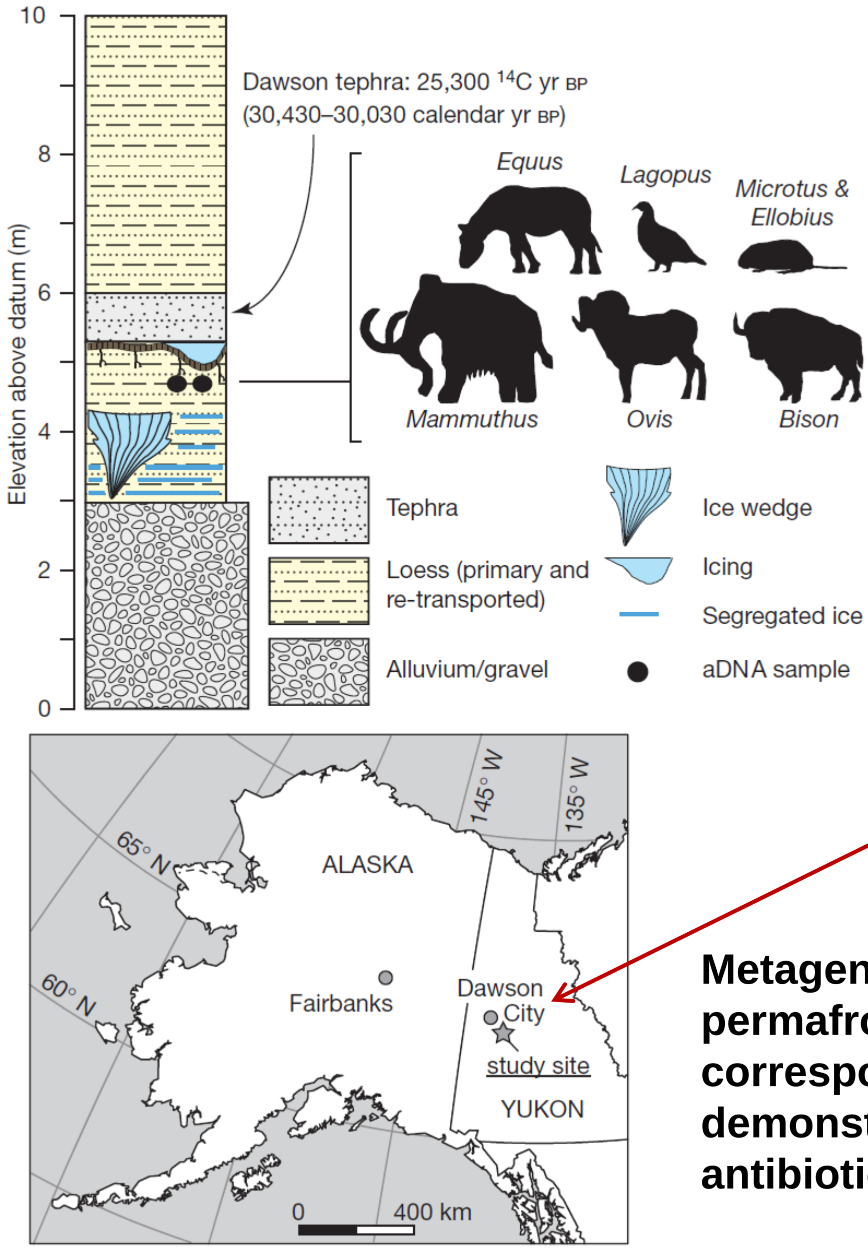
The unexaustable reservoir of antibiotic resistance genes



Schematisches Diagramm der phänotypischen Diversität von Antibiotikaresistenz bei Boden-Bakterien. Es gibt bereits Resistenzen gegen das breit wirksame Tigezyklin, das noch gar nicht therapeutisch eingesetzt wird.

D'Costa, V.M. et al., Science 311, 2006, 374-377

From D`Costa et al.,
Antibiotic resistance is ancient,
Nature, 2011:477: 457.



Dawson City

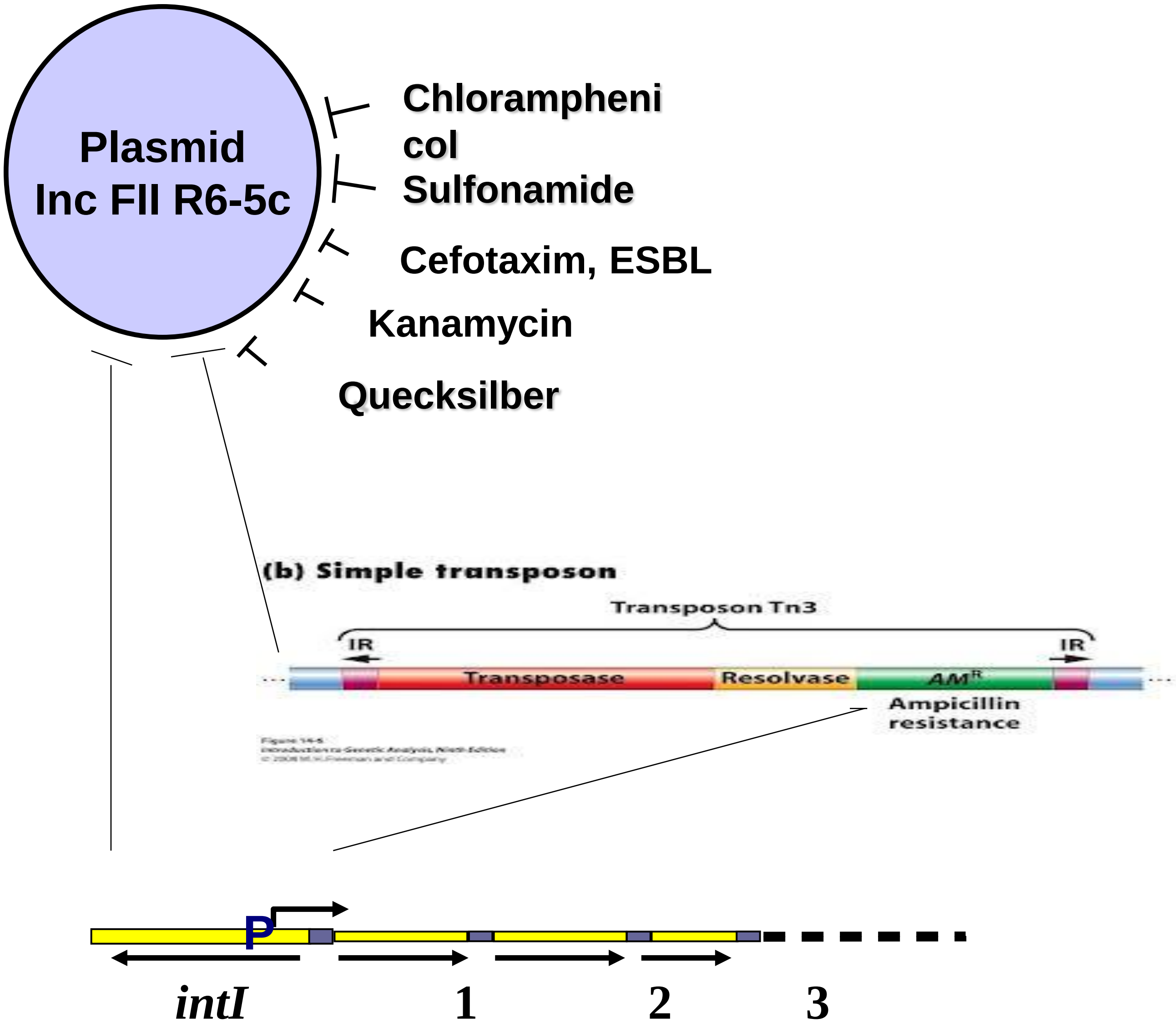
Metagenomic analysis of samples from permafrost soil in Alaska from a depth which corresponds to the age of ~ 27.000 years. demonstration and phylogenetic analysis of antibiotic – resistance genes

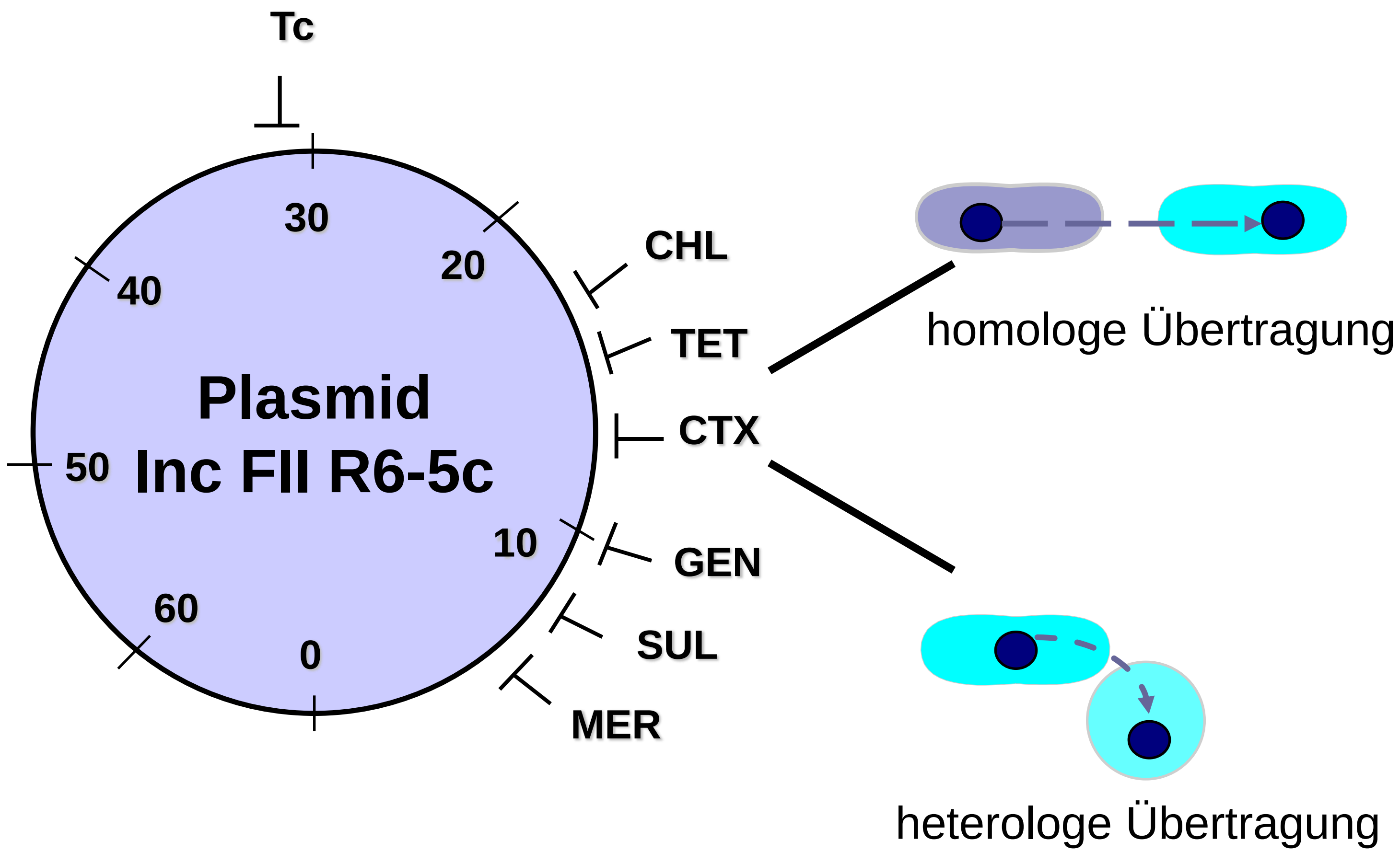
Mobile genetic elements containing antibiotic resistance ge

Plasmids

Transposons

Integrons





Locationof resistance genes on a multiresistance plasmid and transfer to homologous and heterologous bacterial cells.

Resistenzgene: TET gegen Tetracyclin (Transportprotein), CHL gegen Chloramphenicol (Acetylase), CTX gegen Cephotaxim GEN gegen Gentamicin (Phosphorylase), SUL gegen Sulfonamide (Dihydropteroinsäure-Synthetase, MER gegen Quecksilbersalze (Hg-Reductase).

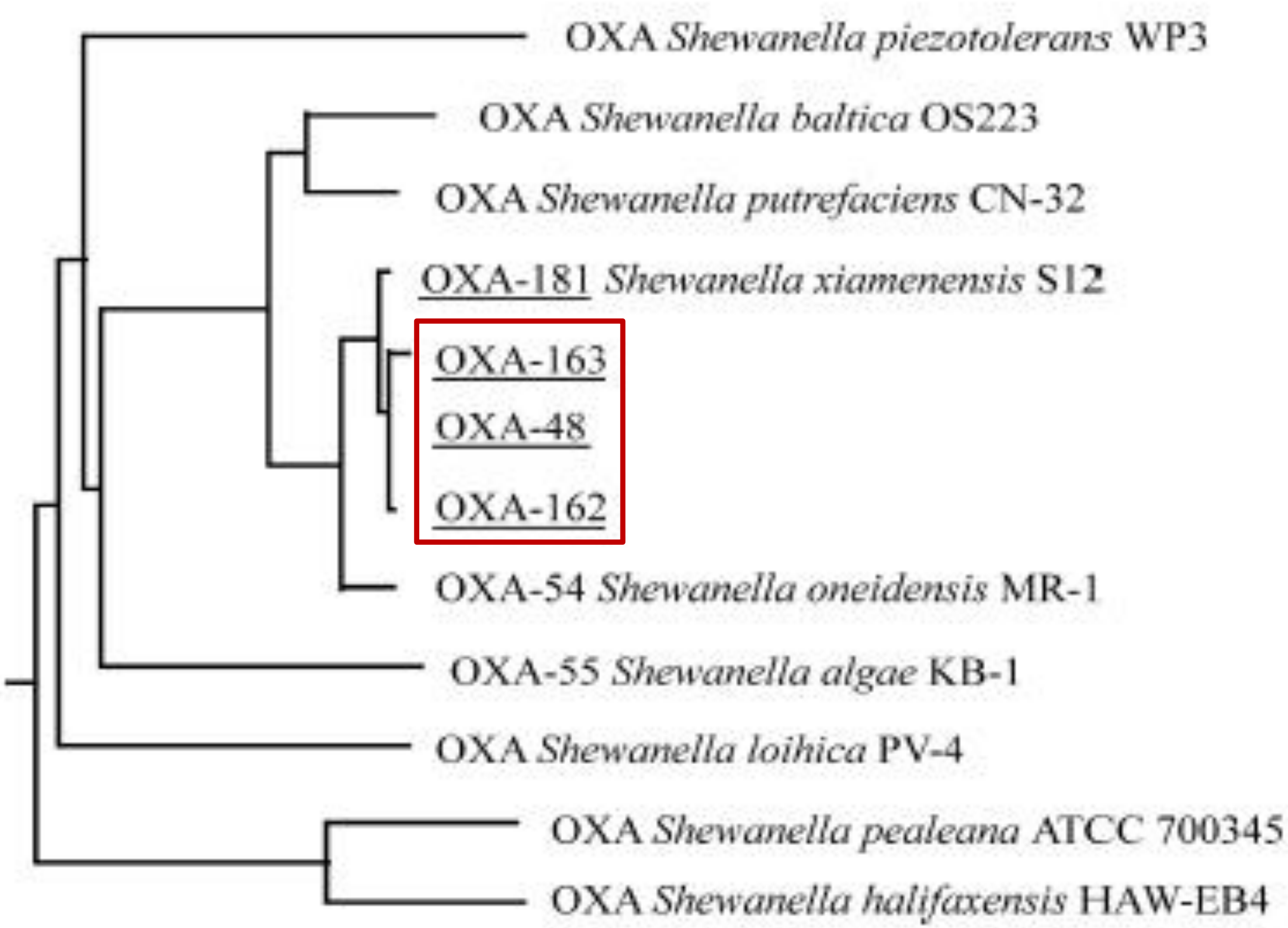
Classical examples for the acquisition of resistance genes by bacteria colonizing and infecting humans and other animals from environmental bacteria

1. Antibiotikc producers: (Julian Davies, Patrice Corvalin)
   - 23SrRNA methyltransferases and MLS –resistance , *erm* -genes
   - Aminoglykoside-modififying enzymes
     (Acetyltransferases, Adenyltransferasen, Phosphotransferases)

2. Environmental bacteria
   - Carbapenemases *(Stenotrophomonas spp., Stenotrophomonas spp.)*
   - ESBL of the CTX-M-type ,(*Kluyvera spp.)*

Relatedness of class-D-carbapenemases from enterobacterial species and from Shewanella spp.

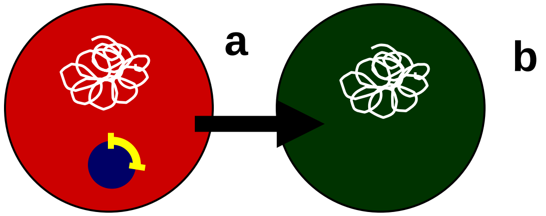
Potron et al. AAC 53;9: 4405-7



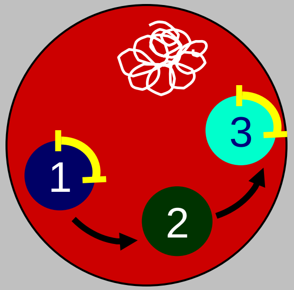
Spread of antibiotic resistance

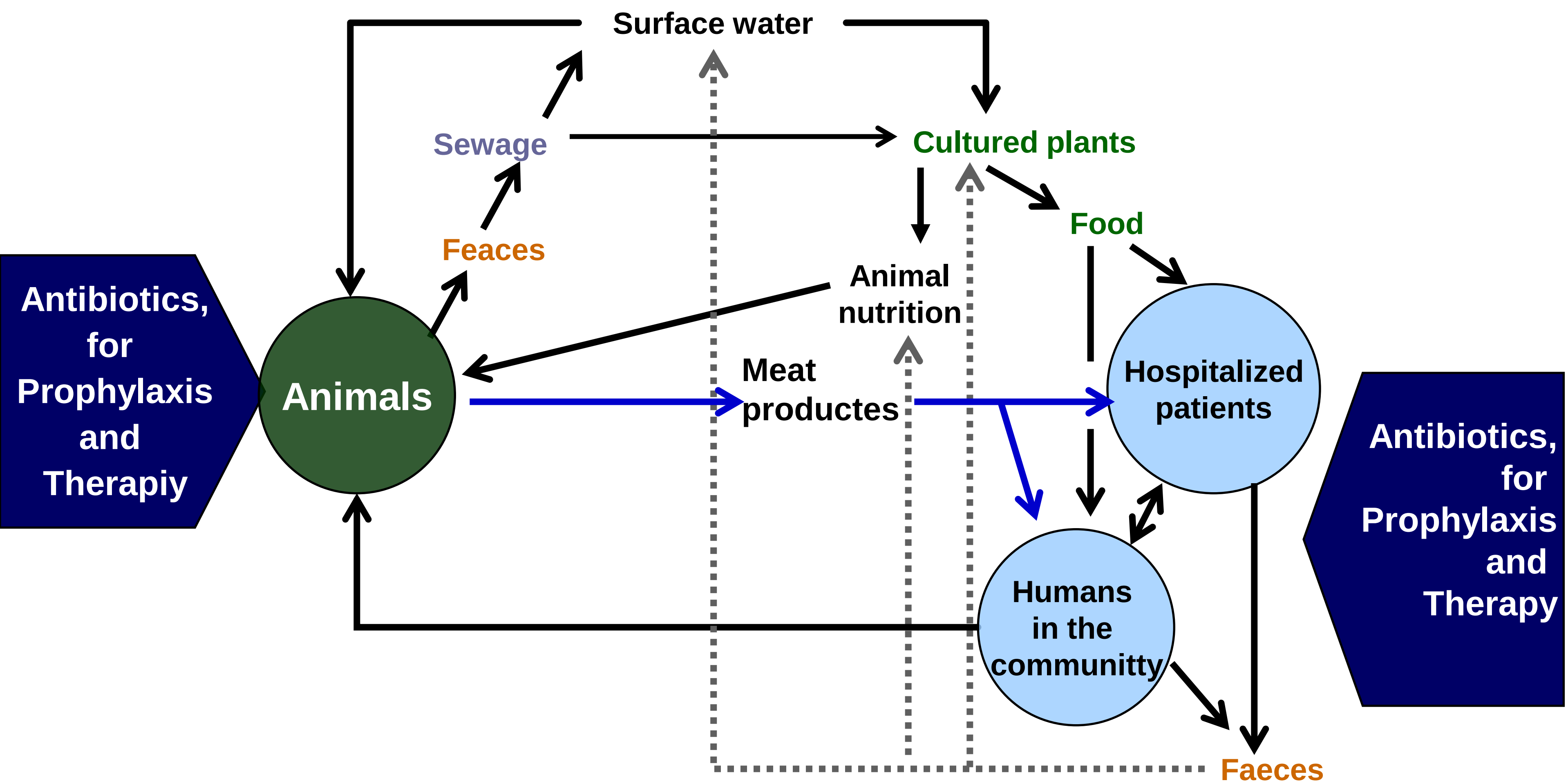
Clonal dissemination of resistant strains
(e,g,. MRSA, resistant *S. pneumoniae*)

Horizontal spread of R-Plasmids
Between different strains



Horizontal spread of resistance genes among Different plasmids

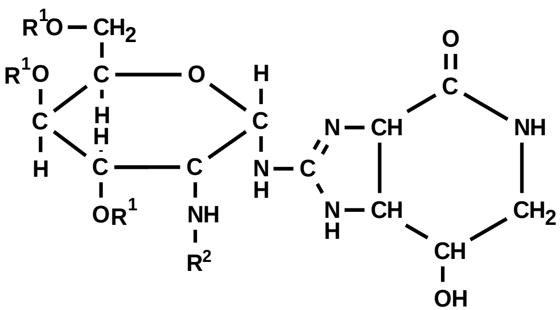




Witte, W., Science; 1998;279(5353):996-7.

Spread of transferable antibiotic resistance btween different ecosystems

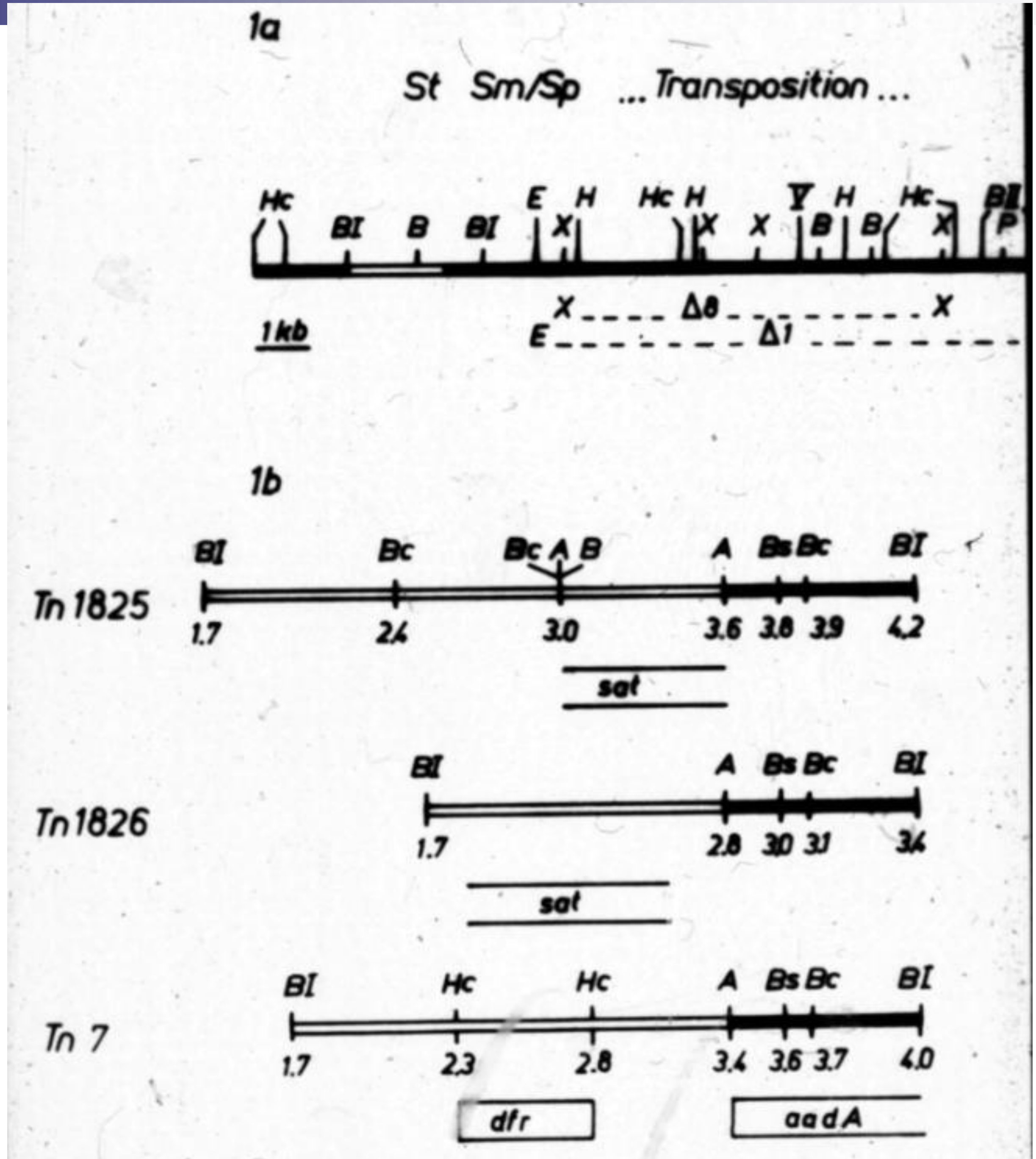
The antibiotic Nourseothricin as groth promoter for livestock



The streptothricin-antibiotic nourseothricin was exclusively used as as growth promoter in conventional livestock from 1980 bis 1989 as replacement for oxytertacycline.There was no use fpor other purposes

The emerging resistance mechanism (Acetyltransferase) did not confer cross resistance to other antibiotics.

The resistance gene (*sat*4*)* is located at a transposon and transferable.


1a
St Sm/Sp ...Transposition...
Hc
BI
B
BI
E
H
X
Hc
H
X
X
Y
H
B
B
Hc
X
BII
P
X
Δ8
X
E
Δ1
1kb
1b
Tn1825
BI
Bc
Bc
A
B
A
Bs
Bc
BI
1.7
2.4
3.0
3.6
3.8
3.9
4.2
sat
Tn1826
BI
A
Bs
Bc
BI
1.7
2.8
3.0
3.1
3.4
sat
Tn 7
BI
Hc
Hc
A
Bs
Bc
BI
1.7
2.3
2.8
3.4
3.6
3.7
4.0
dfr
aadA

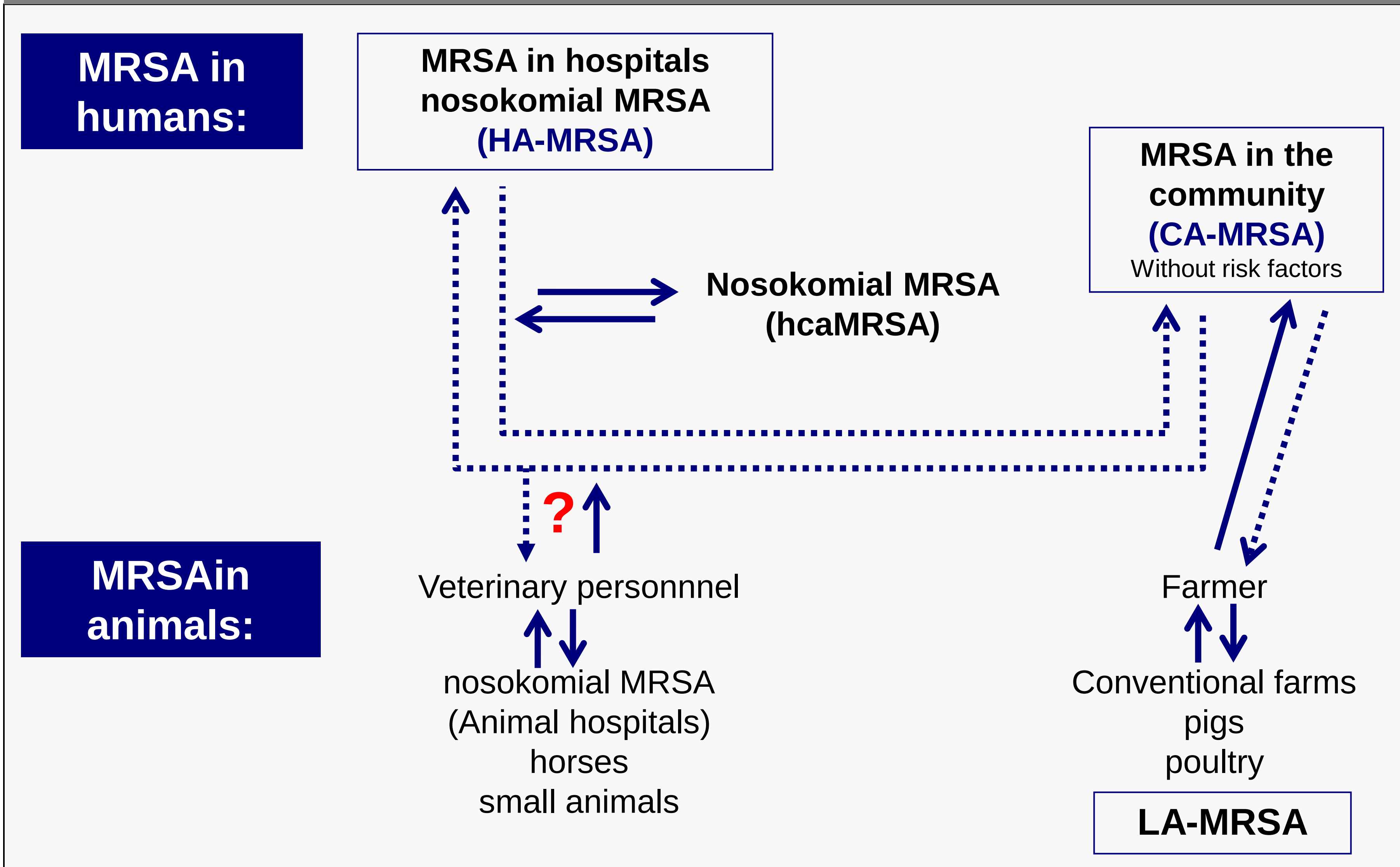
Wide dissemination of sat-1 coded streptothricin resistance (sat-1 in a gene cassette on transposons of Tn*7* family) in former East Germany

	1982	1983	1984	1985	1986	1987
E. coli, faecal flora of nourseothricin fed pigs	-	+	+	+	+	+
E. coli, faecal flora of pig from personnel	-	-	+	+	+	+
E. coli, faecal flora, family members of pig attendants	-	-	+	+	+	+
E. coli, gut flora of healthy adults (also in urban communities)	-	-	-	+	+	+
E. coli, urinary tract infections in humans	-	-	-	+	+	+
Shigella sonnei, diarrhoea in humans	-	-	-	-	-	+

Hummel R, Tschäpe H, Witte W.,J Basic Microbiol. 1986;26(8):461-6.

MRSA: definitions according to epidemiological origin

„Wenn die Begriffe nicht klar sind, breitet sich Unordnung aus“ Konfuzius



Transmision of LA-MRSA to humans

Conventinal farm

Humans with occupational exposition
(farmers, vet`s)
70 – 86 % colonized

Familiar surroundigs

Familiy members
4 - 5% colonized)

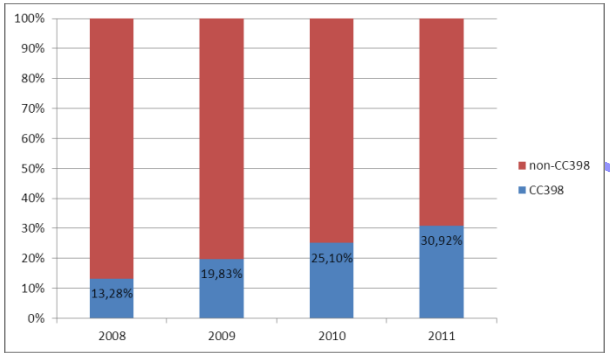
Humans in agricultural areas

Admission to hospitals
2,8% of all MRSA from nosocomial infections in DE)

Cuny, C. et al. PLoS One 2009;27;4(8)e6800

Demonstration of LA-MRSA at hospital admission

Region Münsterland

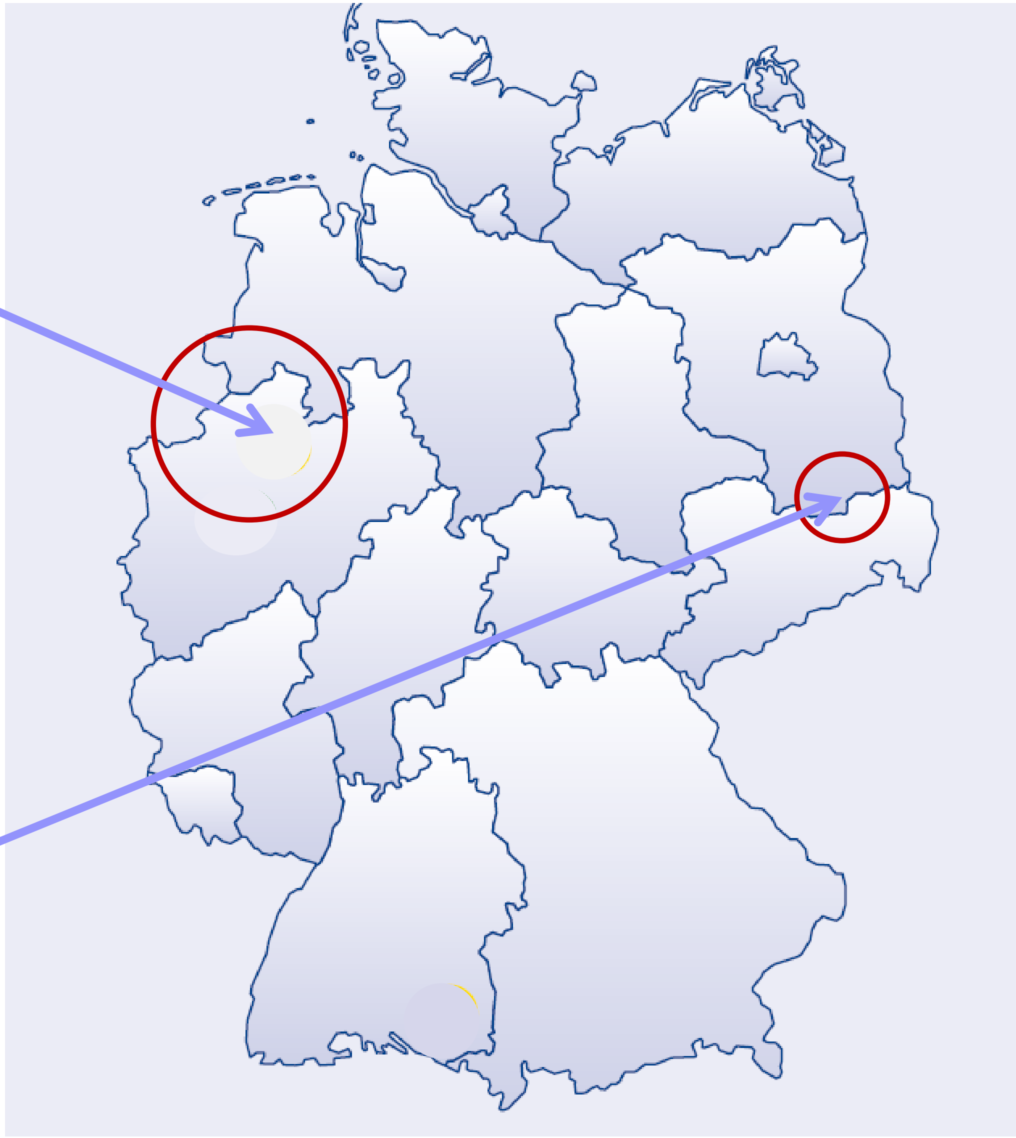


9414 Patienten,
MRSA-Nachweise insgesamt : 1,6 %
Anteil LA-MRSA: ~ 30 %
LA-Nachweise insgesamt: 0.48 %

Köck et al., 2012, Plos One 2013
Schaumburg JCM 2012;

Daten aus dem MRSA-Netzwerk Südbrandenburg (13855 Patienten)

MRSA Nachweise insgesamt: 0.78 %
Anteil an LA-MRSA: 10.8 %
LA-MRSA-Nachweise insgesamt: 0.08 %



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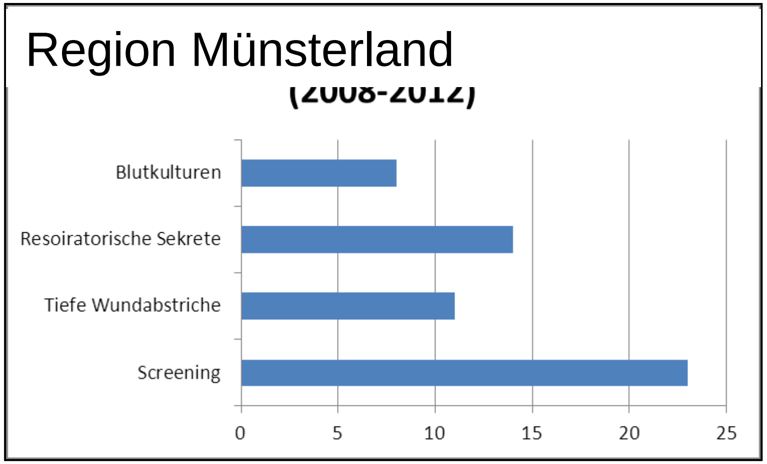
Proportion of LA-MRSA among all MRSA from nosocomial nfections

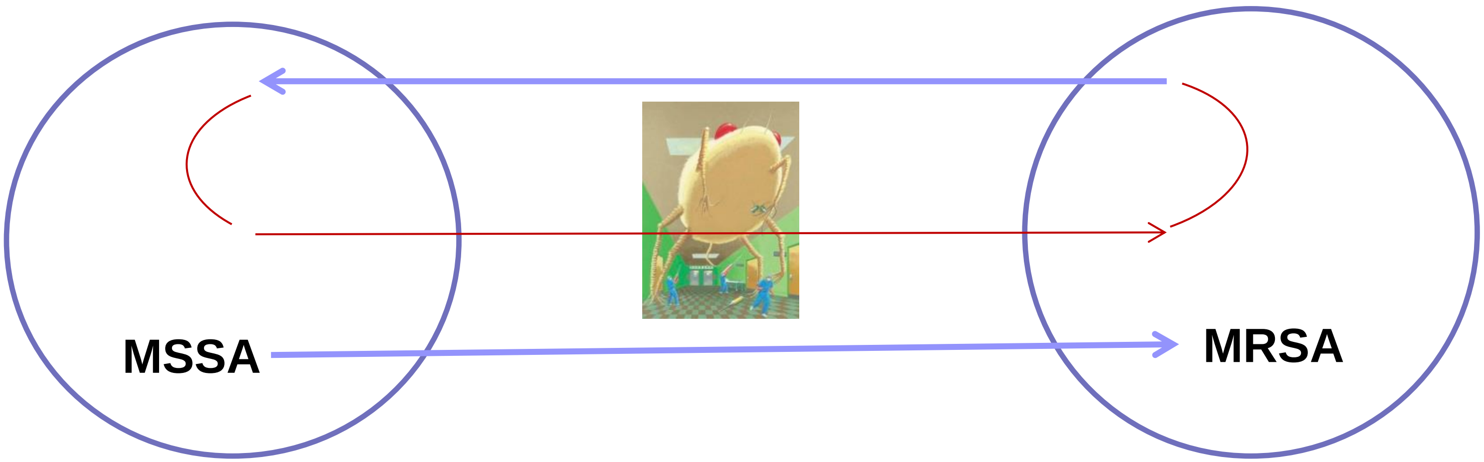


Köck; Plos One 2013
Schaumburg JCM 2012;

Bundesrepublik Deutschland, insgesamt:
2 – 3%, dabei 1,8 % aus Blutkulturen
NRZ f. Staphylokokken u. Enterokokken
Layer et al., Epi.Bull., 2013

STAPHYLOCOCCUS AUREUS /MRSA FROM HUMANS TO ANIMALS AND BACK TO HUMANS

Acquisitiojn of IEC, „readaptation“,2011,
Further virulence genes ?, *luk*-PV ?



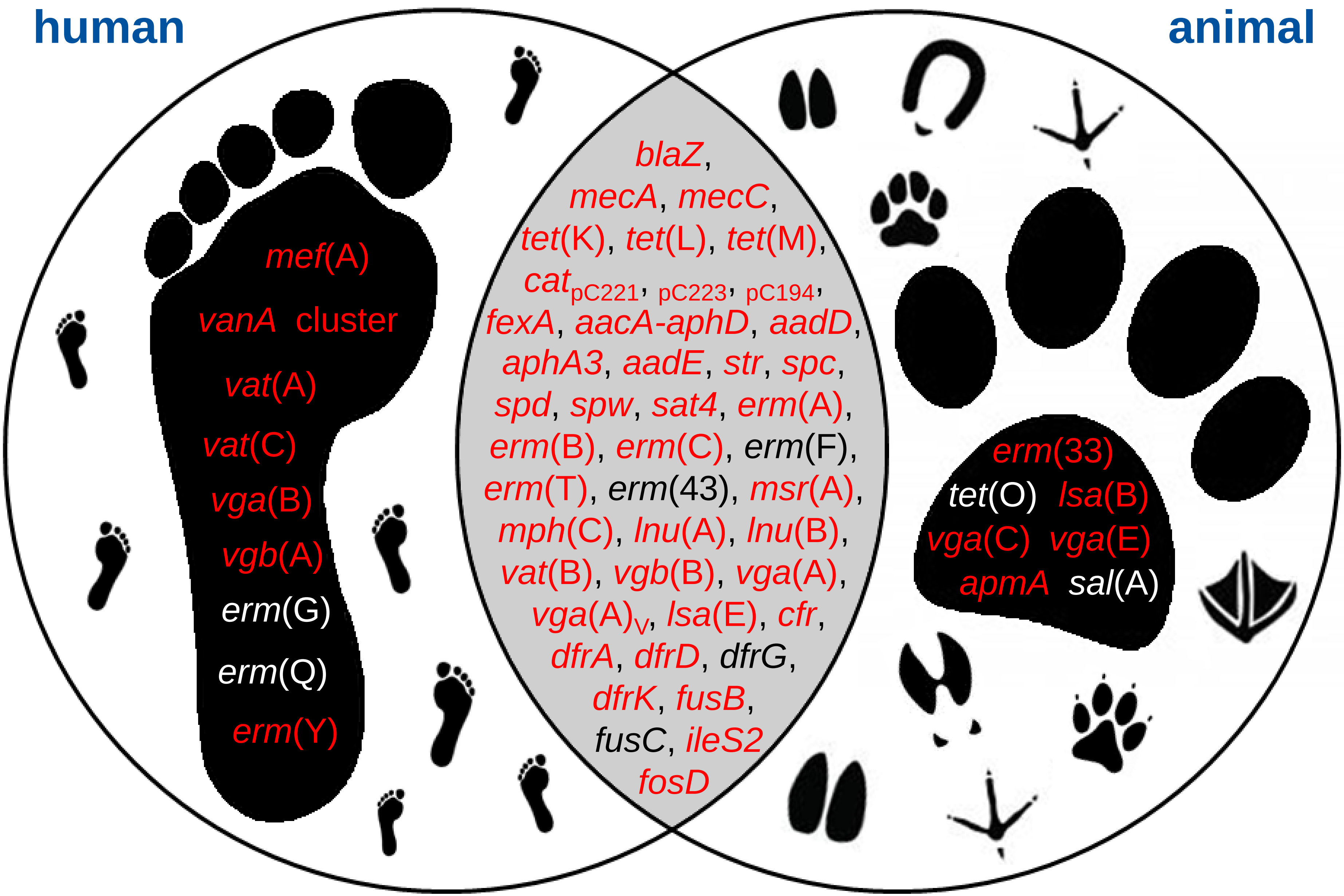
Humans

Pigs , Poultry

End 1990ties

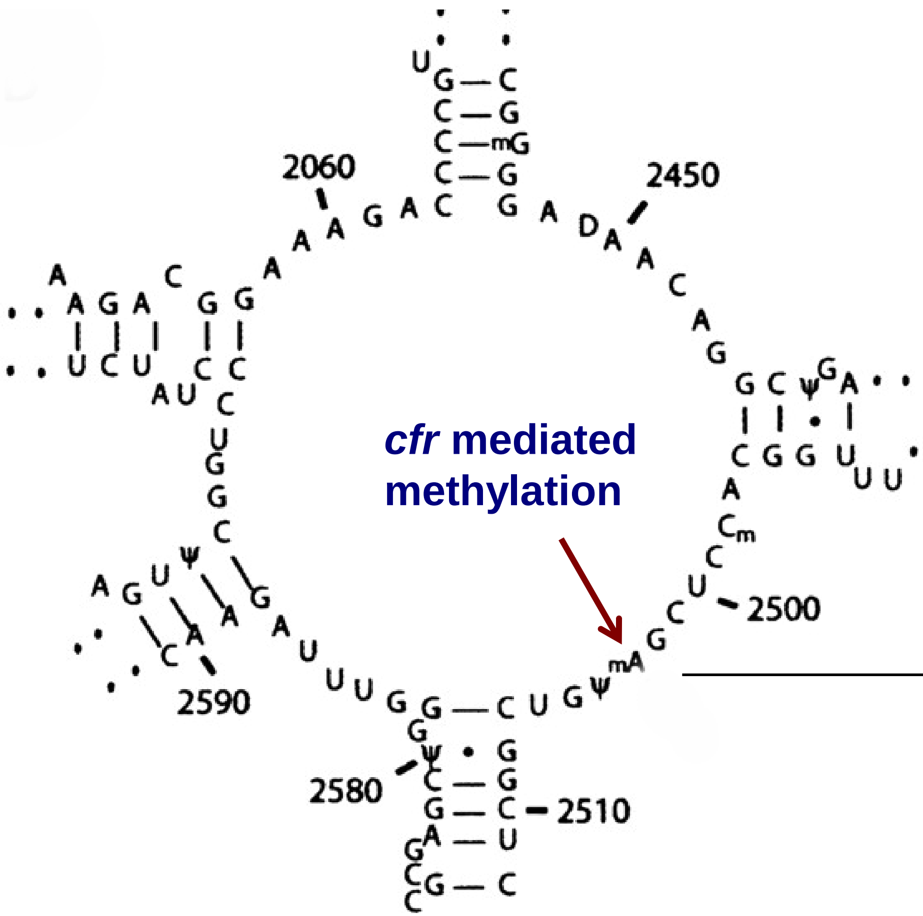
Acquisition vof methicillin resistance
Linked to resistance against
Cu^{2+} und Zn^{2+} , Loss of the
immun evasion gene clusters, IEC

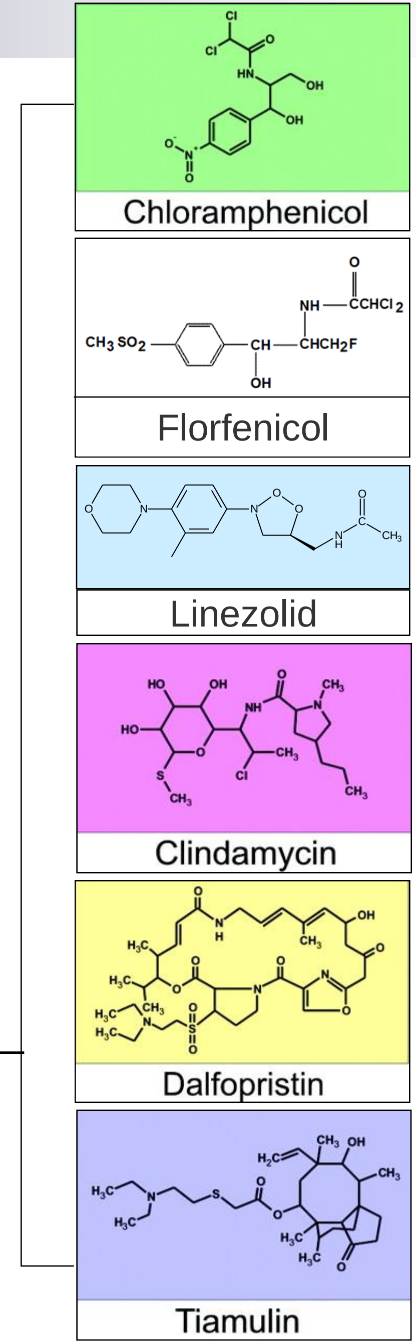
located on mobile genetic elements



Wendlandt, S. et al., Int.J.Med.Microbiol.,2013

Methylation of A2504 by the *cfr* gene encoded methylase mediates cross resistance against several antibiotic classes





Veterinary medicine

Human-medicine

Human-medicine

Human-medicine

Veterinary medicine

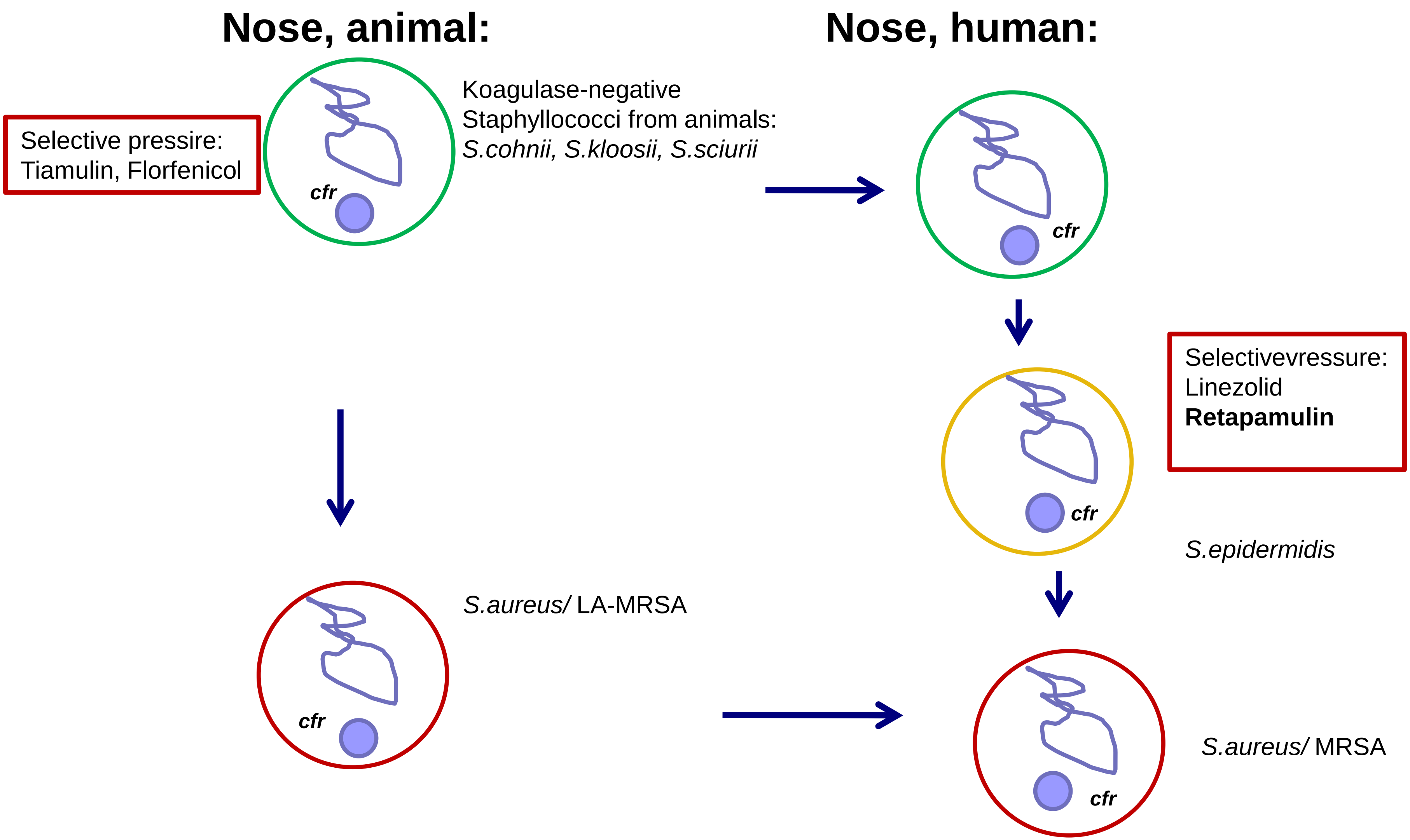
W. Witte; C. Cuny, Future Microbiology, 2011

Resistance to linezolid in *Staphylococcus* spp.; demonstration of *cfr,* samples from Germany

Origin	No. of individuals	
Pig farms, 3 among 8 positive	6 / 67 animals	*S. kloosii, S. cohnii, S. sciuri*
Farmers at these farms	0 / 39	
Veterinarians	4 / 344 (1.2 %)	*S. epidermidis, S. saprophyticus*
Horses		

The plasmid from *S. saprophyticus* was sequenced it is highly homologues (> 98%) to *cfr* containing plasmids from humans in China (*S.cohnii)* , and in the USA (*S.epidermidis, S.aureus)*

Pssibilities for dissemination oththe *cfr*-gene mediated multiresistance against linezolid, pleuromutilines, lincosamidines



Linezolid resistant coagulase negative *Staphylococcus spp*. (CNS) from cattle, L
(cooperation with Dr. Philippe Arnold, Echternsach*)*

Samples from 3 farms from 28 animals (nasal swabs)) 3 farms positive for CNS with
independent upon previous treatment of the animals with florfenicol

Species- distribution :

S.lentus (4)	Conjugation in *S.aureus* 8325-4
S.sciurii (3)	„ „
S.cohnii (1)	„ „
S.equorum (1)	„ „

In a second step investigation (nasal swabs) of 10 humans living on these farms:
no *Staphylococcus spp.* with phenotypic resistance to florfenicol and *cfr*

Colonization and infection with mainly susceptible *S. aureus* ( ) and MRSA ( ), and coagulase negative staphylococci ( )

Vestibulum nasi

Loricrin
Cytokeratin10 ← **ClfB**

Innate immunity,
Compiting flora, e.g.. *S.pneumoniae, Corynebacterium spp.*

Colonizatio n monoclonal

Replacement of resisdent flora by MRSA

very rare under „normal“ conditions

Antibiotics, eg.. Ciprofloxacin

Massive „attack“ “, e.g. dust loaded with MRSA

***S.epidermidis* sometimes co-colonizer**

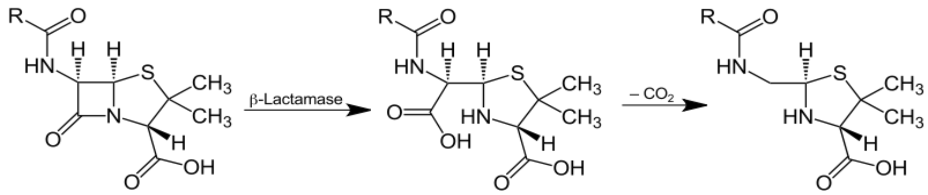
Staphylococcus xylosus
Staphylococcus sciuriii
Staphylococcus cohnii
Staphylococcus saprophyticus
Staphylococcus intermedius

CNS from the nasal flora of livestock

Development of resistance aqgainst ß-Laktamantibiotics in Enterobacteriaceae

1960 **aminobenzyl-penicillines; cephalosporines 1./2. group.**
Ampicillin, Cephalotin

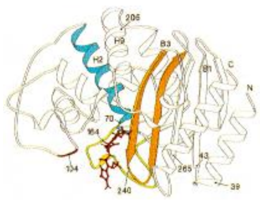
1965 **Emergence of β-Laktamases (penicillinases)**
TEM-1 und SHV-1 (mainly in *E. coli* and *K. pneumoniae*)



1978 **"new" cephalosporins 3. group,** cefotaxim, ceftazidim, cefpodoxim

1985: **Extended-<u>S</u>pectrum <u>B</u>eta-<u>L</u>actamases (ESBL)**

TEM-ESBL,SHV-ESBL

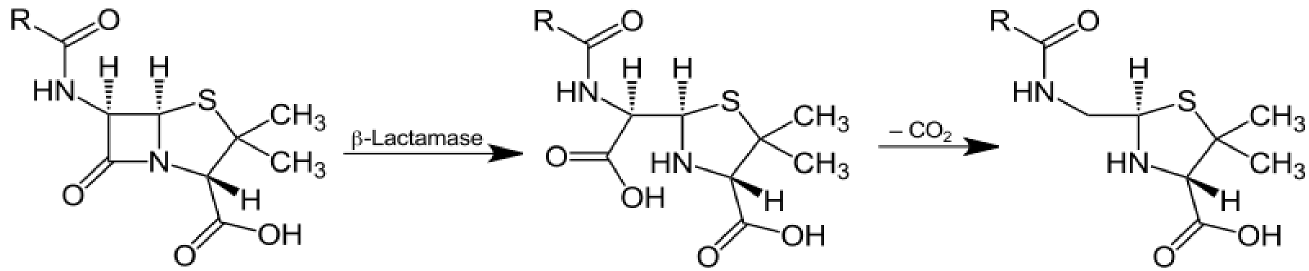


CTX-M-ESBL, origin in *Kluyvera spp.*, acquired by horizontal gene transfer

1985 **Introduction of Carbapenemen** (imipenem, meropenem)

1996: **emergence of carbapenemases**

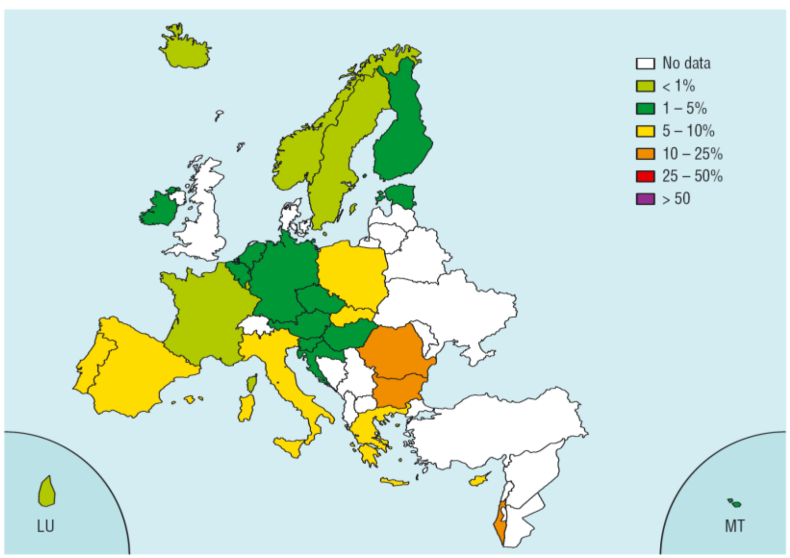
ß-Laktamases in Gram-negative bakteria

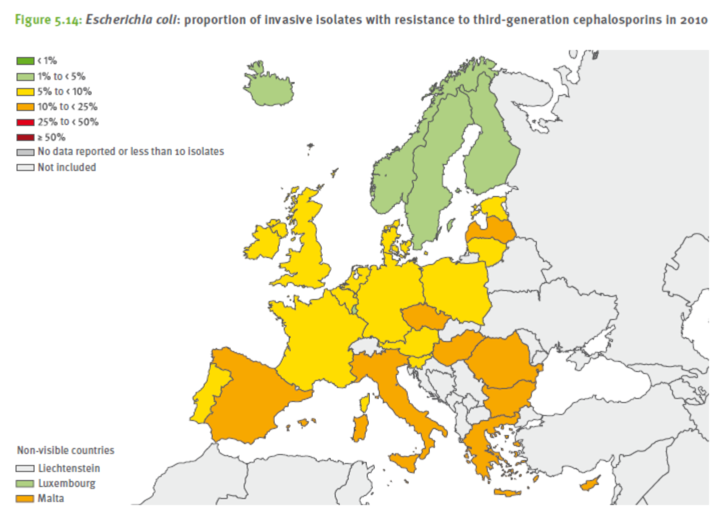


Proportion of *Escherichia coli* with resistance to cephalsosporines of 3rd. generation (ESBL) <u>(EARSS)</u>

2004

2009





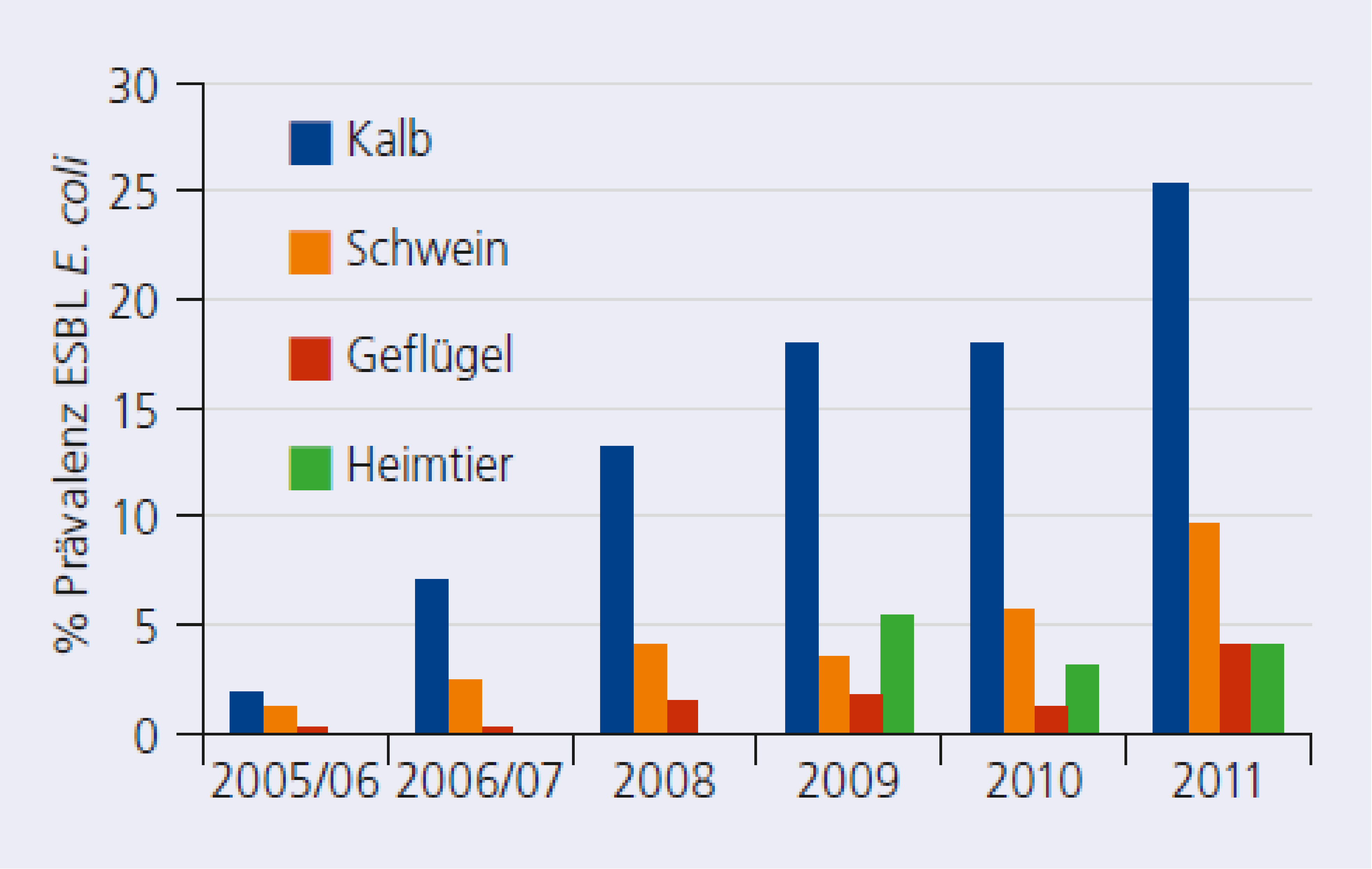
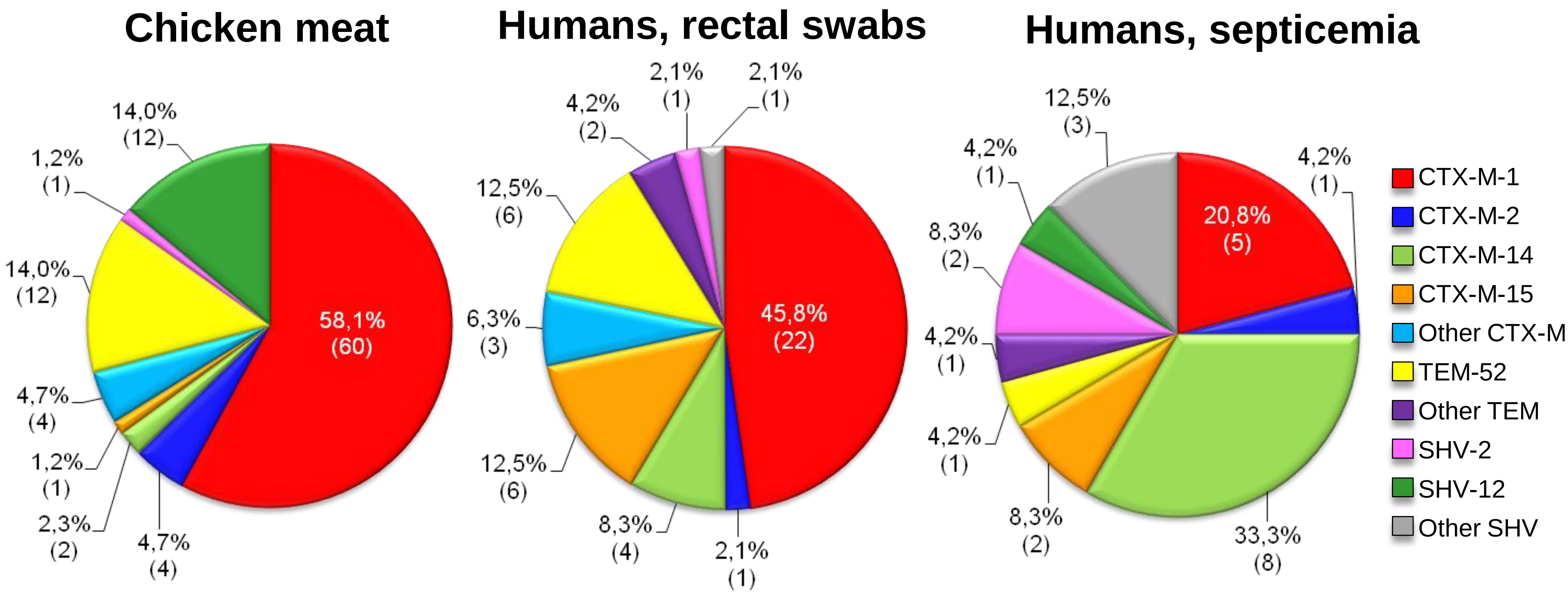


Abb. 1: Prävalenzdaten zu ESBL-bildenden *E.-coli*-Isolaten bei verschiedenen Tierarten, Deutschland 2005–2011

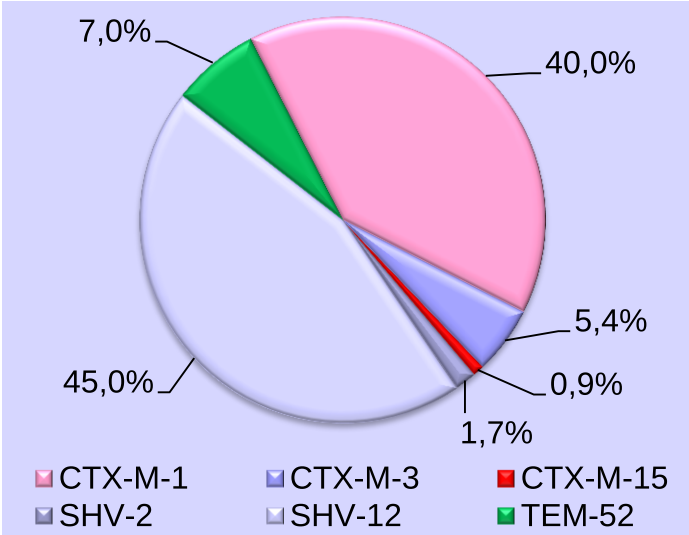
ESBL types in *Escherichia coli* of diferent origin

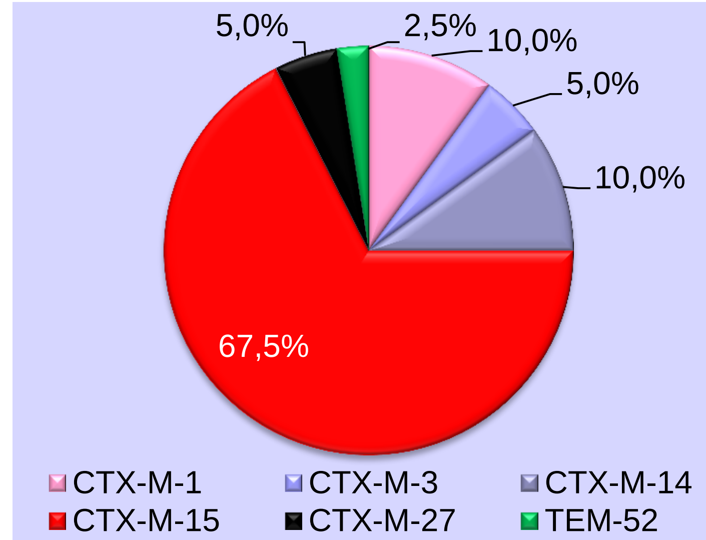
The Netherlands



Distribution of extended-spectrum β-lactamase genes in chicken meat (A), human rectal swabs (B), and human blood cultures (C), the Netherlands. Values in parentheses are no positive.

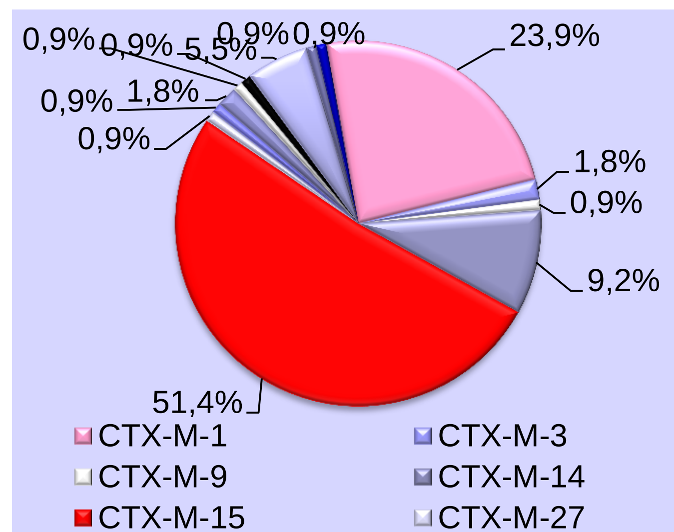
Overdevest et al., EID 2011; 17: 1216-1222





ESBL-types: poultry meat
(Kola et al.,2012)

ESBL-types, humans, ambulatory caret
(GERMAP, 2012)



Types of ESBL genes contained by *E.coli* of different origin Data from Germany

ESBLtypes,, humansnosocomial infections
(GERMAP, 2012)

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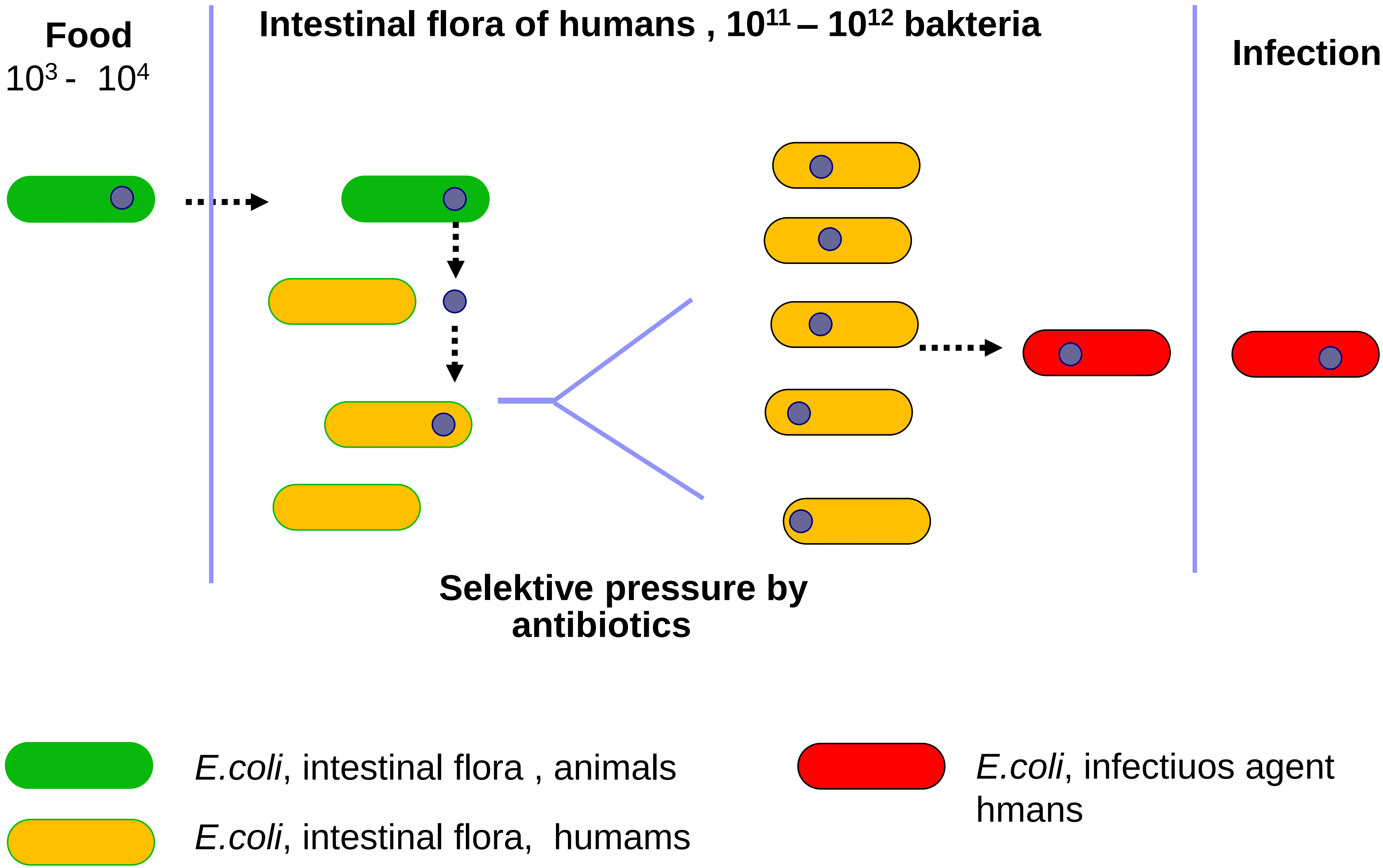
Dissemination of *Escherichia coli* and of its resistance genes from contaminated food to humans as colonizer and as infectious agent



Acquisition of ESBL (*E. coli*) by travelling abroad

Study in the Niehterlands, Paltansing et al., Emerg. Infect. Dis. 2013; 19: 1206-13

Rectal swabs immediately before and after return from 370 persons; (average age: 33 years))

370 persons: 32 positive for ESBL before travel
113 negativ before and positive after travel (30,5%)

Travel to: Soth-East Asia and China for 73% of the positive persons

Durationof travel: ~ 21 days

Most frequent ESBL CTX-M1; n = 22

CTX-M15; n = 16

- no antibiotic usage before and during travel, ;
- no hospital stay abroad;
- besides one individual no travel associated diarrhoea .

Study in Sweden: Tängden et al., AAC, 2010

104 persons; negativ immediately before travel

25% positive at return from: Asia, Africa, Southern Europe

Sammeln von Staub in Tiermastanalgen und in ihrer Umgebung

„Immission“ of LA-MRSA from pig farms in DE

With exhausted air : demonstrated site still i 500 m distance down wind (Schulz et al., ApplEnvironmMicrobiol, 20120

Study on nasal Besiedlung colonization living near to pig farms
Distance of houses < 500 m, frequency of colonization: 1,5 %

Private „Farm-visits“, shopping in form shops: 3,1 % consumers !

In the same sample: humans working in hospitals: 1,9 %

(Study in Lower Saxony Bisdorff et al., Epidemiol.Infect. 2012)

Results from a study in the Netherlands on colo-rectal colonization of humans with ESBL *Escherichia coli* in humans living on countryside in areas with high and low density of chicken farms

Colonization frequency ~ 5.1%, a bit lower (nonsignificant) in areas with low density
The only riks factor: possesssion of horses
Explanatiojn by the authors:: Owners of horses often have other home animals

Huijbers PM, et al. Clin Microbiol Infect. 2013 Jan 7. doi: 10.1111/1469-0691.12150. [Epub ahead of print]

Demonstration of ESBL- Enterobacteriace in dogs, cats, horses in NL
Dierikx et al. J Antimicrob Chemother 2012;67: 1368-74.

Demonstration of ESBL- *E.coli* in dogs
Switzerland: 7,4%, Huber et al. Vet Microbiol 2013;162: 992-6
Tyrol (AU): 5.3% , Franiek et al. Berl.Münch Tierärztl Wochenschr 2012;125: 469-75

Spread of ESBL *E.coli* by sewage sludge and manure

Demonstration in surface water, municipal sewage treatement plant, Styria, Austria Rheintaler et al. Water.Res. 2010;44: 1981-5

Spread of ESBL *E.coli* from cattle
Study in France , A.Hartmann et al., Frontiers in Microbiology,2012;3)

Demonstration of the same isolates (MLST) with the same CTX-M-types
In isolates - from cattle
- from manure
- from soil samples of manured pastures
- demonstration in soil one year after !
(dependend upon quantities of bacteria applied)

Antibiotic resistence gene in soil

Forsberg KJ, et al. Bacterial phylogeny structures **soil** resistomes across habitats. Nature. 2014 May 21. doi: 10.1038/nature13377. [Epub ahead of print]

- Investigation of 18 samples (acre, pasture) on the presence of genes conferring resistance agains t18 antibiotics
- 2895 resistance genes, mainy „new“ genes
- profile of resistance genes different in different samples
- resistance genes limnked to particular taxa, horizontal resistance gene transfer in soil obviously rare
- Higher content of particular resistance genes in manured soil

Contamination of vegetables with ESBL *Eschrichia coli*

Study in the Netherlands: Reuland EA et al. Prevalence of ESBL-producing Enterobacteriaceae in raw vegetables. EurJ Clin MicrobiolInfect Dis, May,2014

- Investigation of 110 samples from 15 different vegetables
- from this 3 samples with *bla* CTX-M15 most frequent in humans

3 samples with *bla* CTX-M15	most frequent in humans
1 sample with *bla* CTX-M1	in humans and livestock
2 samples with *bla* CTX-M9	in humans and animals
1 sample with *bla* SHV	in humans and animals

Study in Germay: vegetariens are colonized with ESBL *E.coli* as humans who "omnivors", Leistner, R. et al. Infect Drug Resist. 2014 Mar 13;7:57-62.

Demonstration of ESBL in 10% of faecal samples of rooks (Corvus frugilegus) in several European countries, also in DE, Literak et al., 2011-2012,
Demonstraion of MRSA and of ESBL in faecal samples of rooks in Ausria
Loncaric;I,et al.:PLoS One. 2013 Dec 31;8(12):e84048. doi: 10.1371/journal.pone.0084048

Rooks are omnivors (seedlings, small animals in soil, in
in urban areas such as in parks also rests of food

Their radius nof action is up to 20 km

They often live in colonies

Spread of ESBL *E.coli* by surface water

Demonstration in the outlet of a sewage treatment plant as well as in rivers in Olsztyn, Poland
(TEM-K, CTX-M, OXA, SHV)
Korzeniewska et al. EcotoxicolEnvironmSaf, 2013

Demonstration in the outlet of a sewage treatmen plant as well as in the river in Barcelona, Spain
(beside others. *E.coli* ST131, CTX-M-15)
Colomer-Lluch *et al.,* J.Antimicrob.Chemother. 2013.

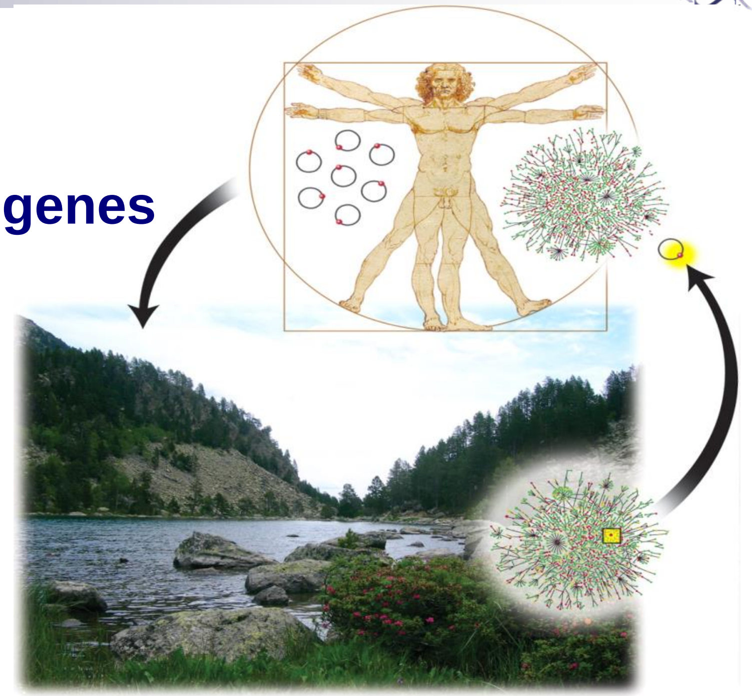
Demonstration in river Thame, West-London
Dhanij et al. J.Antimicrob.Chemother, 2013

Demonstration in 21 from 58 rivers and lakes in Switzerland
(CTX-M-1, **CTX-X-M-15,** CTX-M-27)
Zurfluh et al., Appl.Environm.Microbiol. 2013

Spread by migrating birds

Crane , ESBL, Pacific area Raum
Kitadi et al. J.Vet.Sci.2012;74:395-7

Canada- goose , multiple resistant *E.coli*
Middleton & Ambrose, J.Wildlife Dis. 2005;41: 334-41



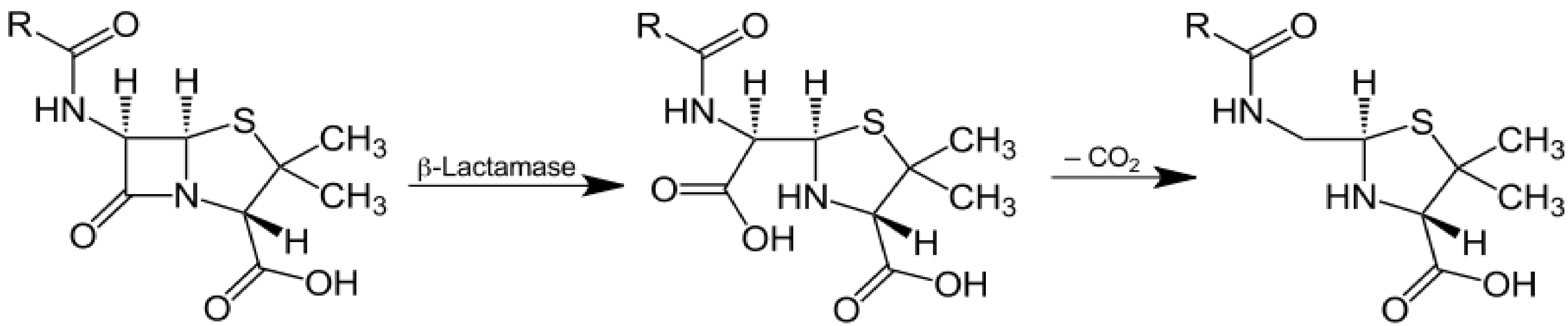
Demonstration of new antibiotic resistance genes in bacteria of the autochtonic microflora of rivers

rare so far

plasmid-located n *qnr in Aeromonas punctata* and *Aeromonas media* from river Seine, Paris
(Cattoir et al., Emerg. Infect. Dis. 2008; 14: 231-237).
Obviously horizontal transfer from *Enterobacteriaceae* to *Aeromonas* spp.,(*qnr* medfiates low level fluoroquinolone resistance by protection of the DANN-gyrase).

***bla*$_{IMI-2}$ (Carbapenemase), located at a 65-kb-plasmid in *Enterobacter asubriae* in several rivers in the USA**
(Aubron et al., Emerg. Infect. Dis. 2005; 11: 260-264)

ß-Laktamases:



ESBL = **E**xtended **S**pectrum **b**eta**-L**aktamases

„Classical“ ß-Laktamases hydrolyze aminopenicillines
and acylureidopenicillines

ESBL possses an exte nded substrate spectrum, they also hydrolyze cephalsporines of groups 3 und 4
ESBL originate from
- Mutations in genes of the classical enzymes
- Acquisition of **ESBL** by horizontal gene transfer from environmental bacteria

Emergence of carbapenmases

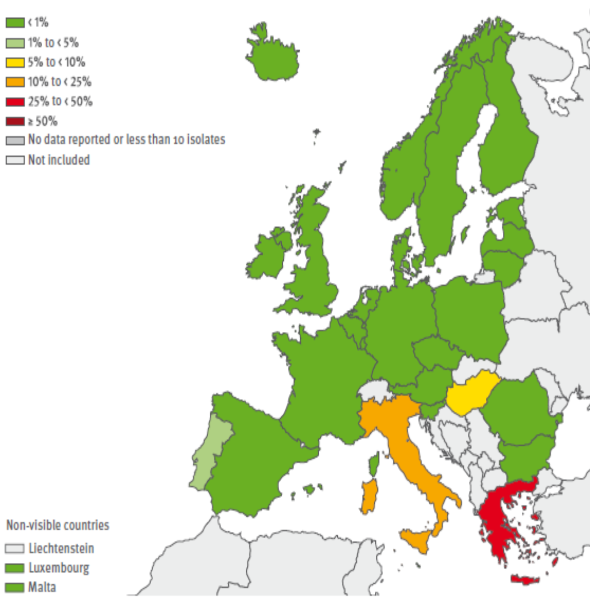
hospitals in DE

↓ sewage ↓ surface water ↓

↓ discharged patients ↓ occupation ↓

↑ Colonization of prospective patients ↑ Meat products ↑

conventional farms in DE in DE ← workers from countroies with high prevalence of carbapenem resistant enterics



Demonstration of *E.coli* containing VIM-2 in 2 farms, and in the outlet of a sewage treatment plant

Summary:

- Multiple antibiotic resistant bacteria are disseminated between humans and livestock in both directions

- Routes of dissemination are professional exposition
  contaminated food
  the environment

- Selective pressure by antibiotics in **both**, human and veterinary medicine favours spread of resistance

- Sided blame is contraproductive, what we need is cooperation !