

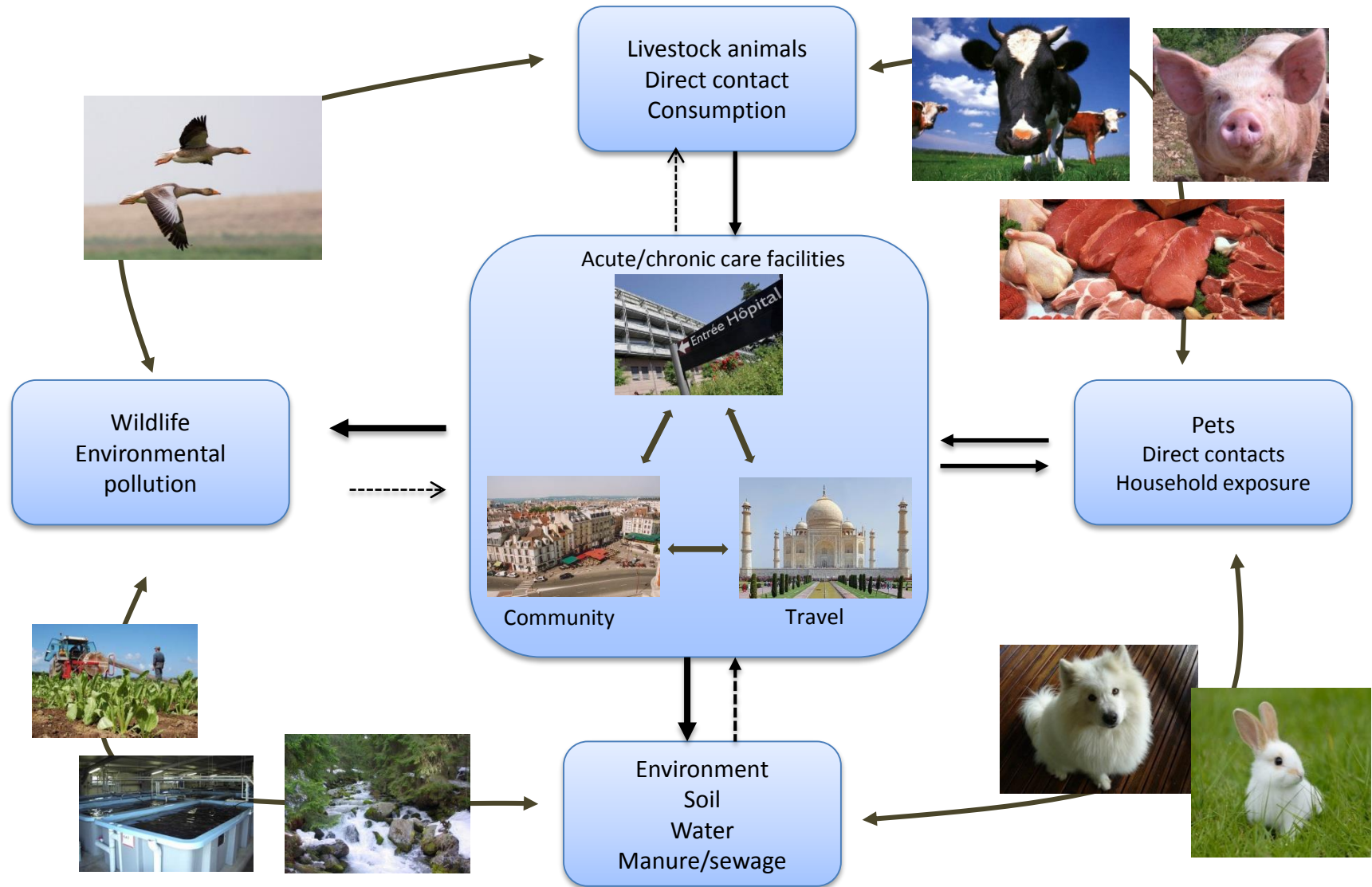


Emergence of new mechanisms of resistance in bacteria isolated from humans and animals

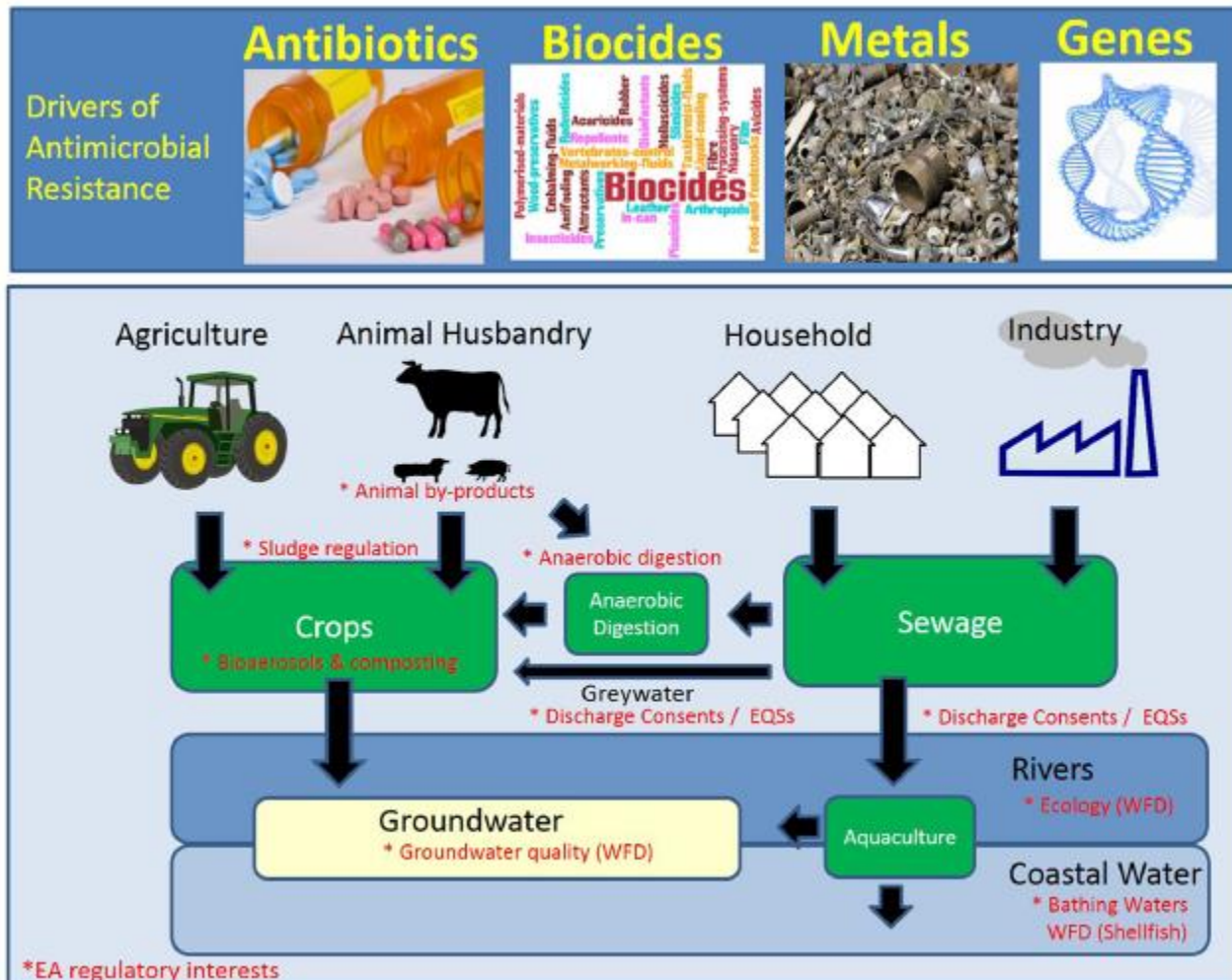
Olivier Denis and Youri Glupczynski



Transmission pathways of antimicrobial resistance among different habitats and ecosystems



Hot-spots and drivers of antimicrobial resistance (AMR)



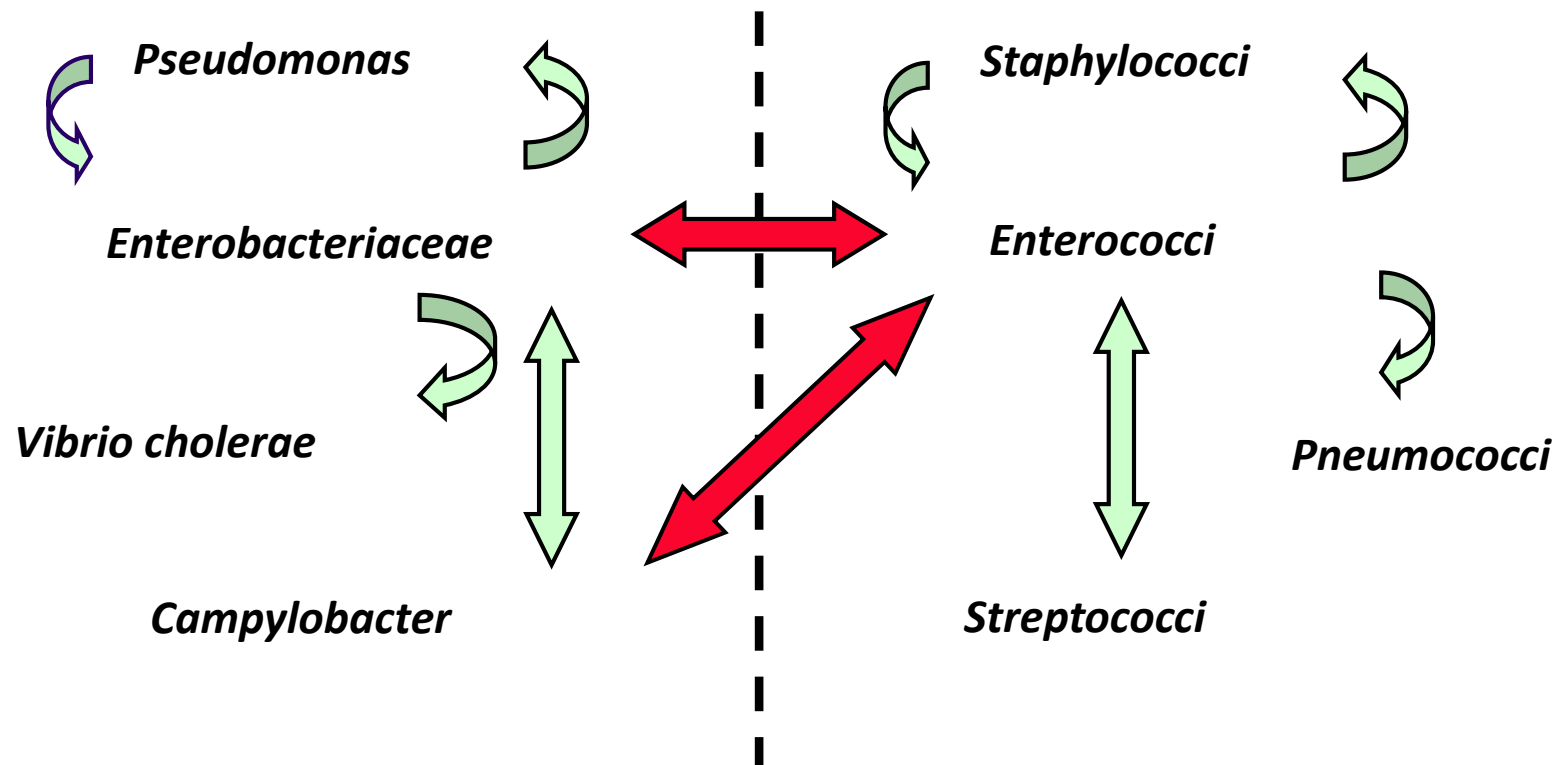
Genetic basis of resistance: Transferable resistance



co-resistance; co-expression; co-selection

Horizontal transfers of resistance genes

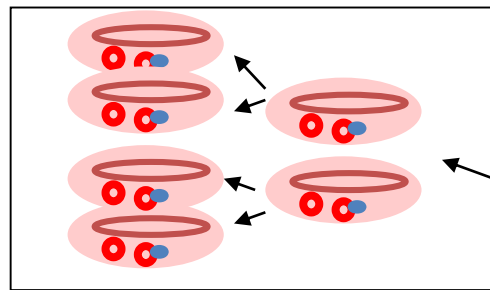
DNA exchange between bacteria belonging to same or different genus/species



Transmission and spread of antimicrobial resistance

- **Horizontal and vertical transmission**

- Vertical transmission by clonal expansion



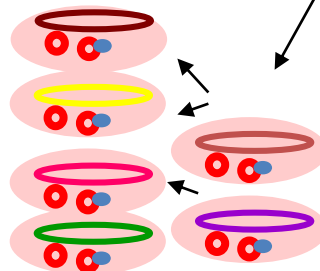
Organisme donneur

Chromosome

Transposon

Plasmide

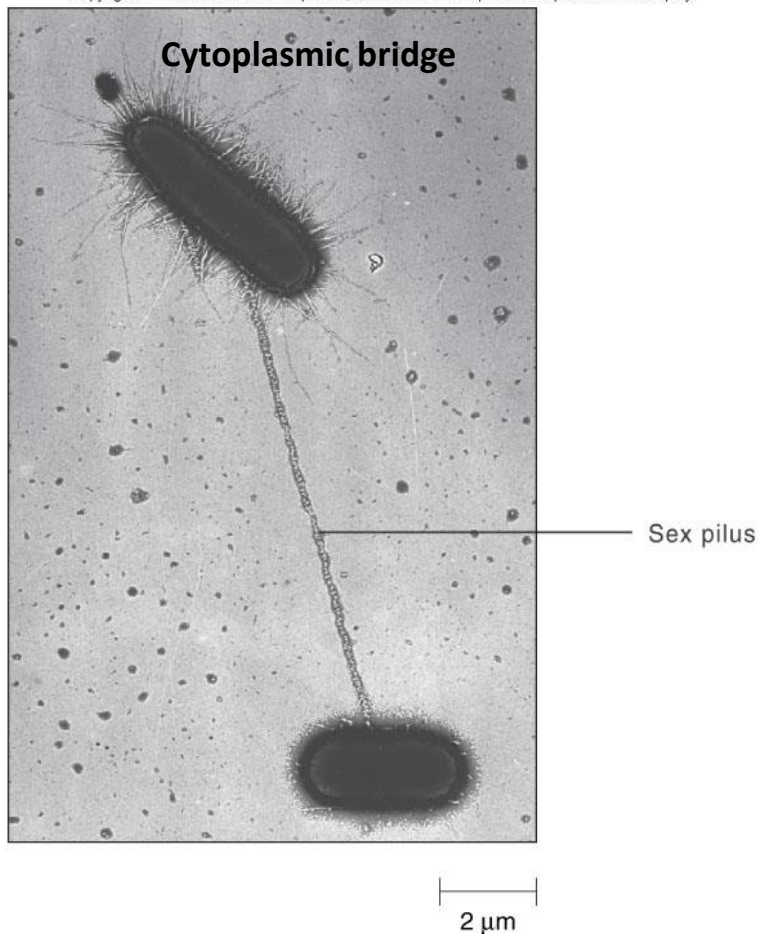
- Horizontal transmission: plasmid mediated conjugation



Transmission and spread of antimicrobial resistance

- **Microscopic and macroscopic transmission**

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The molecular epidemiology of antimicrobial resistance...in a nutshell

The genes move between plasmids (or chromosomes)
...and

The plasmids move between strains, species and genera
...and

The bacteria move between hosts and settings



The challenging multi-drug resistant nosocomial pathogens



Gram-Positive

- *Staphylococcus aureus* (MRSA, GISA, GRSA)
- Glycopeptide-Resistant Enterococci (GRE)

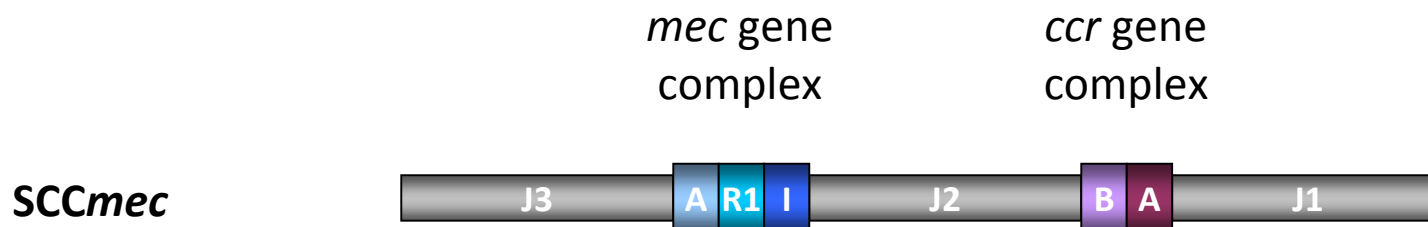
Gram-Negative

- ESBL & carbapenemase producing *Enterobacteriaceae*
 - *Klebsiella* and *Enterobacter*
- Carbapenemase producing & MDR *Pseudomonas*
- MDR *Acinetobacter*

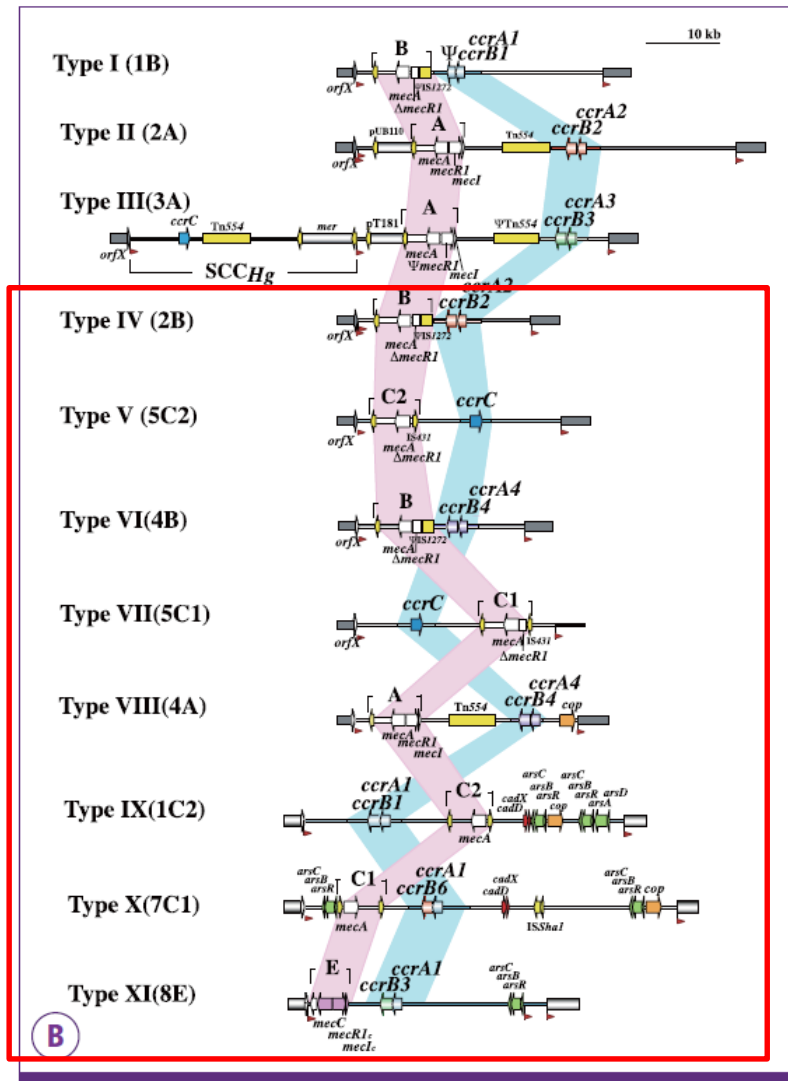
Enterococcus faecium, *Staphylococcus aureus*, *Klebsiella* spp, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp = **ESKAPE**

Methicillin-resistant *S. aureus* (MRSA)

- **Acquisition of *mec* gene encoding PBP2a**
 - PBP2a shows low affinity to β -lactams
 - **Cross-resistance** to all β -lactams, except for the novel anti-MRSA cephalosporins
 - Three different types described: *mecA*, (*mecB*), *mecC*
- **The *mec* gene is integrated into mobile genetic element**
 - Staphylococcal cassette chromosome *mec* (SCC*mec*)
 - Chromosomal insertion at the attB_{SCC} at the end of orfX
 - Often contain plasmids or transposons carrying resistance genes



Staphylococcal Cassette Chromosome *mec*



Classification according to

- **Types:** combination of *mec* and *ccr*
- **Variants:** difference into *junkyard regions*.

SCC _{mec}	Type <i>ccr</i>	Type <i>mec</i>
I (1B)	1	A1B1
II (2A)	2	A2B2
III (3A)	3	A3B3
IV (2B)	2	A2B2
V (5C2)	5	C1
VI (4B)	4	A4B4
VII (5C1)	5	C1
VIII (4B)	4	A4B4
IX (1C2)	1	A1B1
X (7C1)	7	A1B6
XI (8E)	8	A1B3

Staphylococcal Cassette Chromosome *mec*

Type VI(4B)

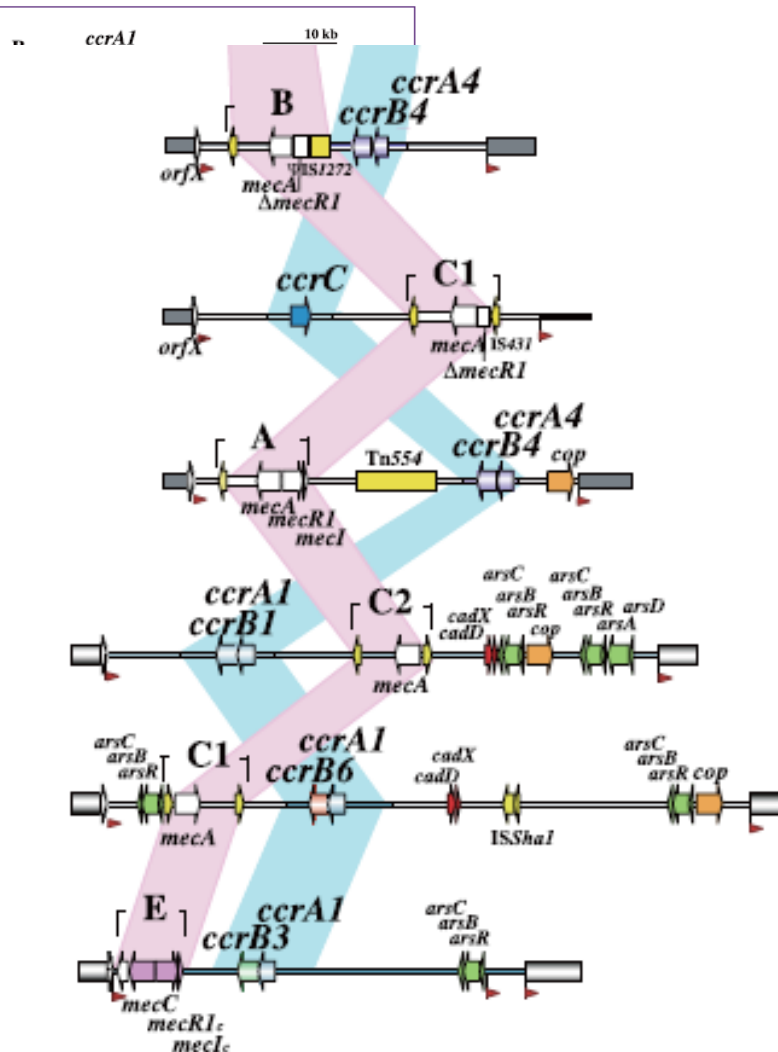
Type VII(5C1)

Type VIII(4A)

Type IX(1C2)

Type X(7C1)

Type XI(8E)



c and ccr
linkyard regions.

ccr	Type <i>mec</i>
1B1	B
2B2	A
3B3	A
2B2	B
1	C2
4B4	B
1	C1
4B4	A
1B1	C2
1B6	C1
1B3	E

MRSA transmission between humans and pets

Human-to-Dog Transmission of Methicillin-Resistant *Staphylococcus aureus*

Engeline van Duijkeren,*
Maurice J.H.M. Wolfhagen,† Adrienne T.A. Box,‡
Max E.O.C. Heck,§ Wim J.B. Wannet,§
and Ad C. Fluit‡

- Transmission within households, veterinary hospitals and farms
 - Dogs, cats but also rabbits, parrots, ... horses
 - HA-MRSA
 - ST22-SCC*mec* IV, ST8-SCC*mec* II
 - Transmission of nurse to her baby and dog
 - PVL- positive CA-MRSA
 - ST80-SCC*mec* IV, USA300 ST8-SCC*mec* IV
 - Familial outbreaks with recurrent infection

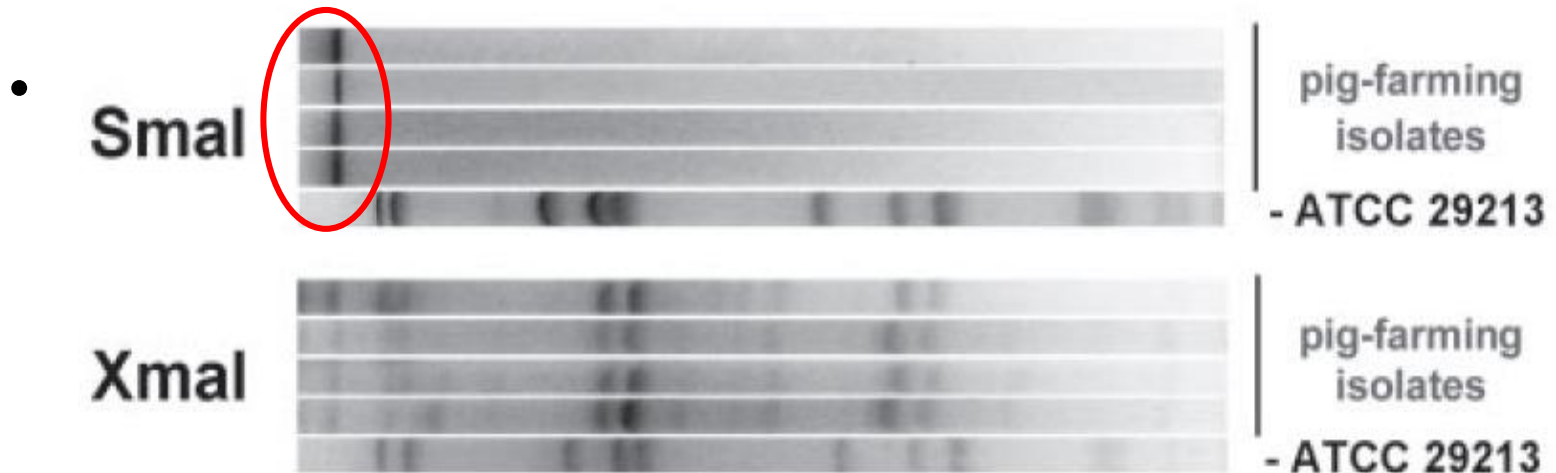


Rankin S. et al. Vet Microb. 2005:108
van Duijkeren E. et al. JCM. 2005:43
Vitale CB et al. EID 2006:12
van Duijkeren E. et al. EID 2004:10
Strommenger B. et al. JAC 2006:57

**Methicillin-
resistant
*Staphylococcus
aureus* in Pig
Farming**

MRSA and livestock animals

Andreas Voss,*† Frans Loeffen,* Judith Bakker,*
Corne Klaassen,† and Mireille Wulf*



- **Netherlands**
 - Unexpected cases of MRSA in population without known risk factor and correlated with pig-farming or with pigs
 - 6/26 farmers (23%) colonized with MRSA
 - All MRSA strains were not typeable by *SmaI* PFGE analysis
 - By MLST all isolates belonged to **ST398**

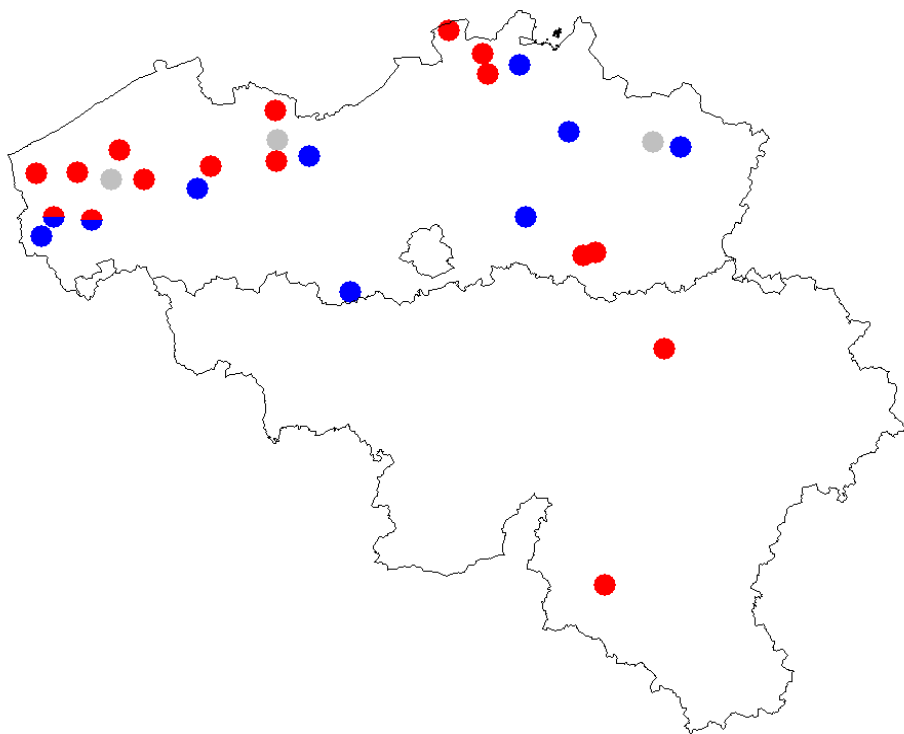


Spread outside the hospital environment)

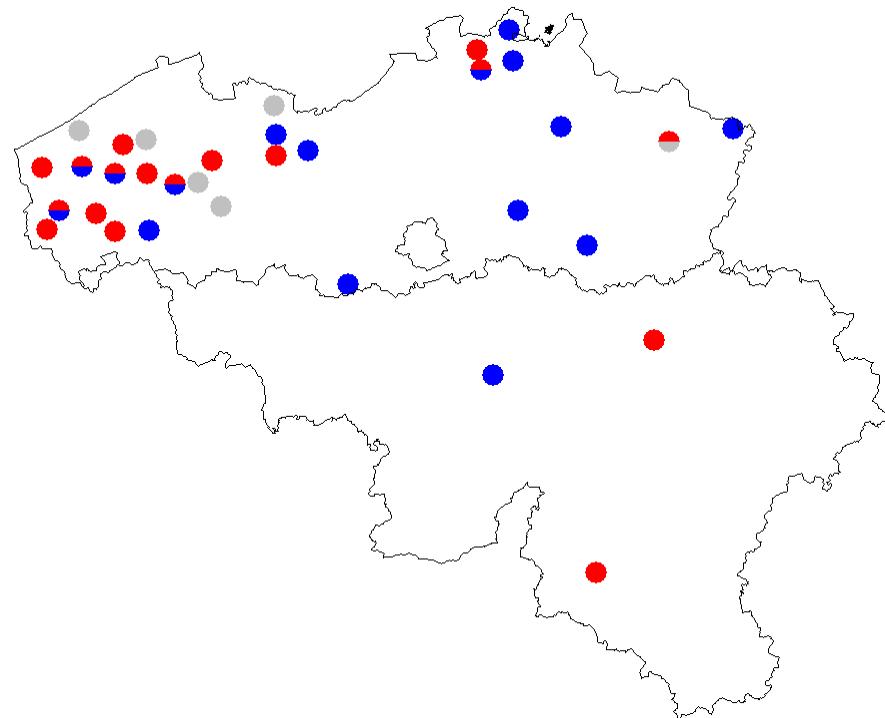
- **Almost monoclonal belonging to CC398**
 - Highly frequent in Europe but also in the USA
 - In Asia ST9
 - Acquisition of the *SCCmec V* but also IV, "VII" new variants (IX, X)
- **Often multi-resistant**
- **High prevalence in some human populations**
 - Veal farmers (58%), pig farmers (38%), Veterinarians (7.5%) (Belgium)

Distribution of LA-MRSA CC398

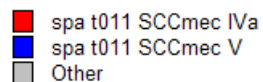
Farmers versus pigs, 2007



Farmers



Pigs



MRSA carriage among veterinarians in Belgium and Denmark

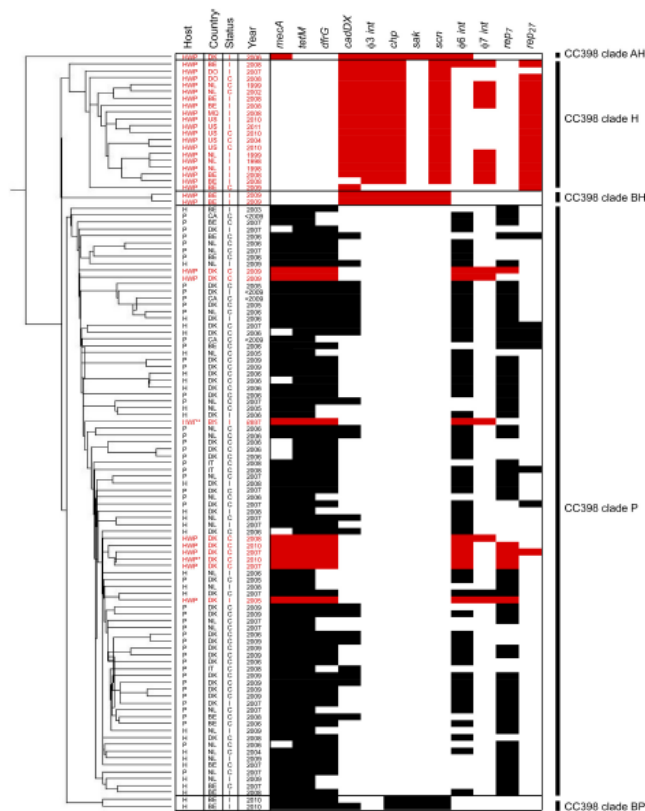
Exposure variable	No. (%)		OR (95 % CI)	P value*
	Belgium	Denmark		
All veterinarians	(n = 146)	(n = 143)		
MRSA ST398	11 (7.5)	2 (1.4)	5.7 (1.2–54.0)	0.01
MRSA non-ST398	3 (2.1)	0 (0)		
Total MRSA	14 (9.6)	2 (1.4)	7.5 (1.7–68.6)	0.0023
Veterinarians working with livestock	(n = 105)	(n = 97)		
MRSA ST398	11 (10.5)	2 (2.1)	5.6 (1.2–52.6)	0.02
MRSA non-ST398	2 (1.9)	0 (0)		
Total MRSA	13 (8.9)	2 (2.1)	6.7 (1.5–62.4)	0.0052
Veterinarians not working with livestock	(n = 41)	(n = 46)		
MRSA ST398	0 (0)	0 (0)		
MRSA non-ST398	1 (2.4)	0 (0)		
Total MRSA	1 (2.4)	0 (0)		

OR, Odds ratio; CI, confidence interval.

* Significant exposure variables are shown in bold (based on a *P* value of ≤ 0.05).

Staphylococcus aureus CC398 Clade Associated with Human-to-Human Transmission

Alex J. McCarthy,^a Willem van Wamel,^b Stien Vandendriessche,^c Jesper Larsen,^d Olivier Denis,^c Cristina Garcia-Graells,^c Ann-Catrin Uhlemann,^e Franklin D. Lowy,^e Robert Skov,^c and Jodi A. Lindsay^a



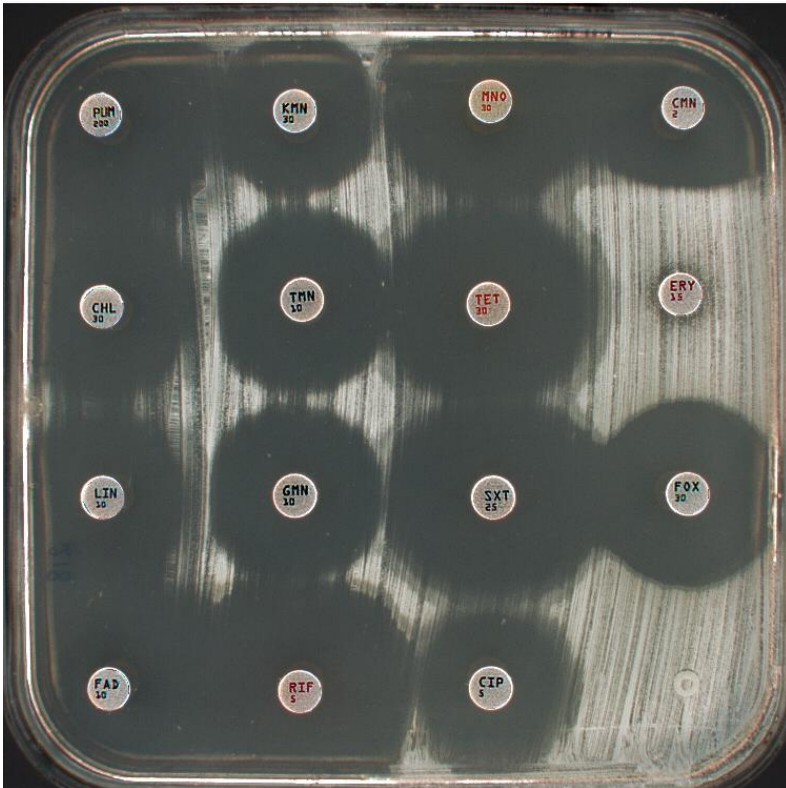
- Human clade associated with bacteriophage $\phi 3$
 - Immune Evasion Cluste
 - Absence of *tetM* gene encoding tetracycline resistance
 - MSSA
- LA-MRSA
 - MRSA
 - *tetM* gene
 - Bacteriophage $\phi 7$

Livestock-associated CC398 MRSA



- **Multi-resistant**
 - To antibiotics
 - Tetracycline: *tetM*, *tetK*, *tetL*,...
 - Aminoglycosides
 - MLS: *ermA*, *ermC*, *ermT*,..
 -
 - To heavy metals (Zn,..)
- **Absence of gene encoding toxins**
 - PVL, TSST-1
 - enterotoxin negative

Human CC398 MSSA



- *mecA* negative
- Particular genotype t571
- MLS_B inducible phenotype
 - Encoded by *ermT* which is an unusual cause of macrolide resistance
 - First described in staphylococci from bovine or porcine origin
- Found in humans without livestock contact
- Responsible of human infections

Methicillin-susceptible *Staphylococcus aureus* ST398-t571 harbouring the macrolide–lincosamide–streptogramin B resistance gene *erm*(T) in Belgian hospitals

Stien Vandendriessche^{1,2*}, Kristina Kadlec³, Stefan Schwarz³ and Olivier Denis¹

¹Laboratoire de Référence MRSA-Staphylocoques, Service de Microbiologie, Université Libre de Bruxelles, Hôpital Erasme, Brussels, Belgium; ²Veterinary and Agrochemical Research Center (VAR), Brussels, Belgium; ³Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), Neustadt-Mariensee, Germany

*Corresponding author. Service de Microbiologie, Hôpital Erasme, 1070 Brussels, Belgium. Tel: +32-2-555-69-71; Fax: +32-2-555-31-10; E-mail: stien.vandendriessche@ulb.ac.be

Received 19 May 2011; returned 23 June 2011; revised 25 July 2011; accepted 27 July 2011

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA), collected from 109 Belgian acute-care hospitals during a national survey in 2008, were investigated for macrolide–lincosamide (ML) resistance with particular emphasis on the analysis of *erm*(T)-carrying isolates.

Methods: In total, 314 MRSA and 212 MSSA were collected and characterized by *spa* typing. The SCCmec type of MRSA was determined. Resistance to ML antibiotics was detected by agar dilution and resistant strains were screened by PCR for *erm*(A), *erm*(C) and *msr*(A). Five ML-resistant MSSA isolates, negative by PCR for the aforementioned genes, were further characterized.

Results: Half of all MRSA isolates ($n=157$; 50.0%) were resistant to erythromycin and harboured the gene *erm*(A) ($n=112$), *erm*(C) ($n=41$), *erm*(A)+*erm*(C) ($n=3$) or *msr*(A) ($n=1$). The *erm*(A) gene was mainly present in MRSA *spa*-CC002-ST5-SCCmec II and *spa*-CC008-ST8-SCCmec IV (where CC stands for clonal complex and ST stands for sequence type); the distribution of *erm*(C) was more diverse. Thirty-five of the 40 erythromycin-resistant MSSA (18.9%) carried the gene *erm*(A) ($n=17$), *erm*(C) ($n=9$) or *msr*(A) ($n=9$). The remaining five MSSA were ST398-t571 isolates, which exhibited closely related ApaI PFGE patterns, harboured the gene *erm*(T) in the chromosomal DNA and did not exhibit additional resistances. These isolates were from severe infections in patients, of whom four had no contact and one had only indirect contact with livestock via a family member working in animal husbandry.

Conclusions: The ML–streptogramin B ('MLS_B') resistance genes *erm*(A) or *erm*(C) were detected in the majority of ML-resistant MRSA and MSSA isolates. The *erm*(T) gene was identified in MSSA ST398 isolates from five independent patients who lacked direct contact with livestock.

Meticillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: a descriptive study

Laura García-Álvarez, Matthew T G Holden, Heather Lindsay, Cerian R Webb, Derek F J Brown, Martin D Curran, Enid Walpole, Karen Brooks, Derek J Pickard, Christopher Teale, Julian Parkhill, Stephen D Bentley, Giles F Edwards, E Kirsty Girvan, Angela M Kearns, Bruno Pichon, Robert L R Hill, Anders Rhod Larsen, Robert L Skov, Sharon J Peacock, Duncan J Maskell, Mark A Holmes

- Unusual MRSA clones harboring *mecC* gene into SCC*mec* XI
- Belonging to clone CC130, CC705, ST425
- Reported in the UK, Denmark, Ireland, Germany, France... and Belgium
- Isolated from various animals
 - Bovine but also dog, rabbit, rat, seal, sheep, chaffinch
 - Causing mastitis
- Isolated from humans causing SSTI, arthritis, bacteremia or asymptomatic carriage
- Problems of detection
 - Low level resistance to oxacillin and cefoxitin
 - No detection by usual PCR targeting *mecA*

Epidemiology and host range

mecC MRSA found in multiple host species across Europe



Genetic diversity among methicillin-resistant *Staphylococcus aureus* isolates carrying the *mecC* gene in Belgium

Ariane Deplano*, Stien Vandendriessche, Claire Nonhoff and Olivier Denis

National Reference Centre – *Staphylococcus aureus*, Department of Microbiology, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium

*Corresponding author. Tel: +32-2-5556971; Fax: +32-2-5553110; E-mail: ariane.deplano@erasme.ulb.ac.be

Received 12 November 2013; returned 9 December 2013; revised 15 January 2014; accepted 19 January 2014

Objectives: A *mecA* homologue gene, named *mecC*, has been reported in methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from humans and from diverse animal species. We investigated the proportion, and the phenotypic and genotypic characteristics, of *mecC*-carrying MRSA recovered from humans in Belgium.

Methods: A total of 4869 *S. aureus* isolates, collected by the National Reference Centre from 2003 to 2012, were retrospectively analysed for the presence of *mecC*. The *mecC*-carrying MRSA isolates were tested for phenotypic resistance and the presence of toxin genes. Genotyping was performed using *spa* typing and multilocus sequence typing.

Results: Nine *S. aureus* isolates, *mecA* negative but cefoxitin resistant (MIC 16–64 mg/L), were found to carry the *mecC* gene. Among these, eight showed resistance to oxacillin (MIC 4–64 mg/L). These isolates remained fully susceptible to all non- β -lactam antimicrobials. Although the proportion of *mecC*-carrying MRSA in Belgium was low (<1% per year), *mecC*-MRSA were assigned to three distinct genetic lineages corresponding to clonal complex (CC) 130, CC49 and CC1943.

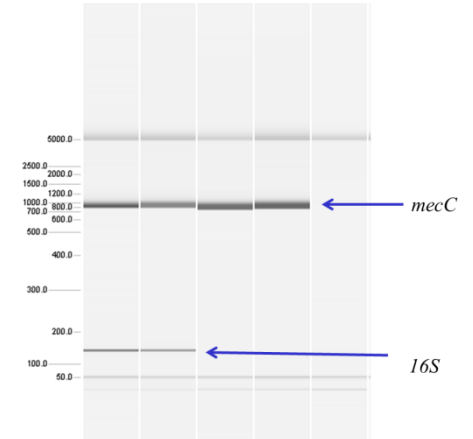
Conclusions: This first Belgian nationwide analysis showed a low occurrence of *mecC*-MRSA. Further studies should be conducted to better understand the reservoirs and risk factors for *mecC*-MRSA acquisition.

mecC - MRSA



CMI oxacilline : 6 mg/l
CMI céfoxitine : 24 mg/l

Positive PCR *mecC*



Linezolid resistance

- Two mechanisms of resistance
 - Mutations in domain V of 23S rRNA (G2576T) or other genes encoding ribosomal proteins
 - **Methylation of nucleotide A2503 = transferable mechanism**
 - *cfr* gene located on plasmids
 - PhLOPS_A resistance phenotype
- Described in *Staphylococcus aureus* and CoNS isolates from animals and humans including Belgium
- Resistance: rare (<1%) but outbreaks occurred

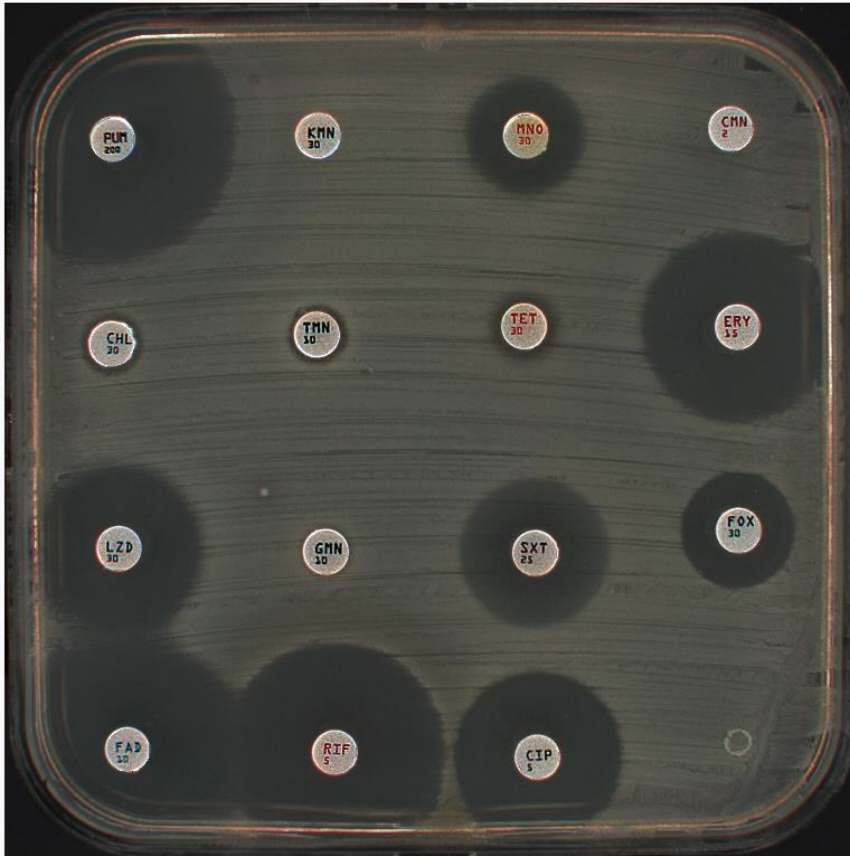
Vanderhaeghen W, et al. JAC 2012
Diaz L et al. AAC 2012
Morales G et al. CID 2012
Sánchez García M et al. JAMA 2010

Clinical Outbreak of Linezolid-Resistant *Staphylococcus aureus* in an Intensive Care Unit

Miguel Sánchez García, MD, PhD

Context Linezolid resistance is extremely uncommon in *Staphylococcus aureus*.

cfr-Positive MRSA ST398



- LA-MRSA ST398
- Resistance to chloramphenicol and clindamycin
- Linezolid susceptible ?
- Not detected by disk diffusion using CLSI guidelines
- MIC to linezolid = 12 mg/L

Emergence of cfr-positive *S. aureus* in Belgium

- Humans
 - 1464 *S. aureus* isolates from 2013 to 2015 sent by 167 laboratories
 - 30 resistant to chloramphenicol, clindamycin and/or linezolid
 - One cfr-positive MRSA belonging to CC398 collected from patient with SSI
 - Linezolid MIC = 12 mg/l
- Animals
 - Occasionally found in *S. aureus* and non *S. aureus*
 - Pigs, veals

Characterization of methicillin-resistant non-*Staphylococcus aureus* staphylococci carriage isolates from different bovine populations

Wannes Vanderhaeghen^{1,2*}, Stien Vandendriessche¹⁻³, Florence Crombé^{1,2}, Stéphanie Nemeghaire¹, Marc Dispas¹, Olivier Denis³, Katleen Hermans², Freddy Haesebrouck² and Patrick Butaye^{1,2}

Results: The MRNAS (n=101) carriage rate was estimated as 30.29% (95% CI 6.14%–74.28%) in veal calves, 13.1% (95% CI 1.28%–63.72%) in dairy cows and 24.8% (95% CI 11.97%–44.42%) in beef cows. Carriage rates were not significantly different between the three populations ($P > 0.05$). *mecA*_{LGA251} was not detected. Most (n=80) MRNAS were identified as *Staphylococcus sciuri*, *Staphylococcus lentus* or *Staphylococcus fleurettii*. Resistance to aminoglycosides, macrolide, lincosamide, streptogramin, antimicrobials, tetracycline, and ciprofloxacin was frequently detected. Two linezolid-resistant MRNAS from veal calves carried the multidrug-resistance gene *cfr*. SCCmec cassettes of type III predominated (n=46); another 40 SCCmec cassettes harbored a class A *mec* complex without identifiable *ccr* complex, type IVa, type V and several other non-typeable cassettes were detected in low frequencies, especially in methicillin-resistant *Staphylococcus epidermidis*.

Conclusions: The SCCmec types predominating in bovine MRNAS differ from those mostly detected in livestock-associated methicillin-resistant *S. aureus* strains. Yet, the detection of *cfr* and the high level of other antimicrobial resistances suggest a potentially important role of bovine MRNAS as a reservoir for resistance determinants other than SCCmec.

Paridaens H et al. submitted

Vanderhaeghen W et al. JAC 2013

Peeters LEJ et al. Vet Microbiol 2015

Angeles Argudin M et al Res Vet Science 2015



Potentially transferable linezolid resistance in *Enterococcus faecium* in the UK

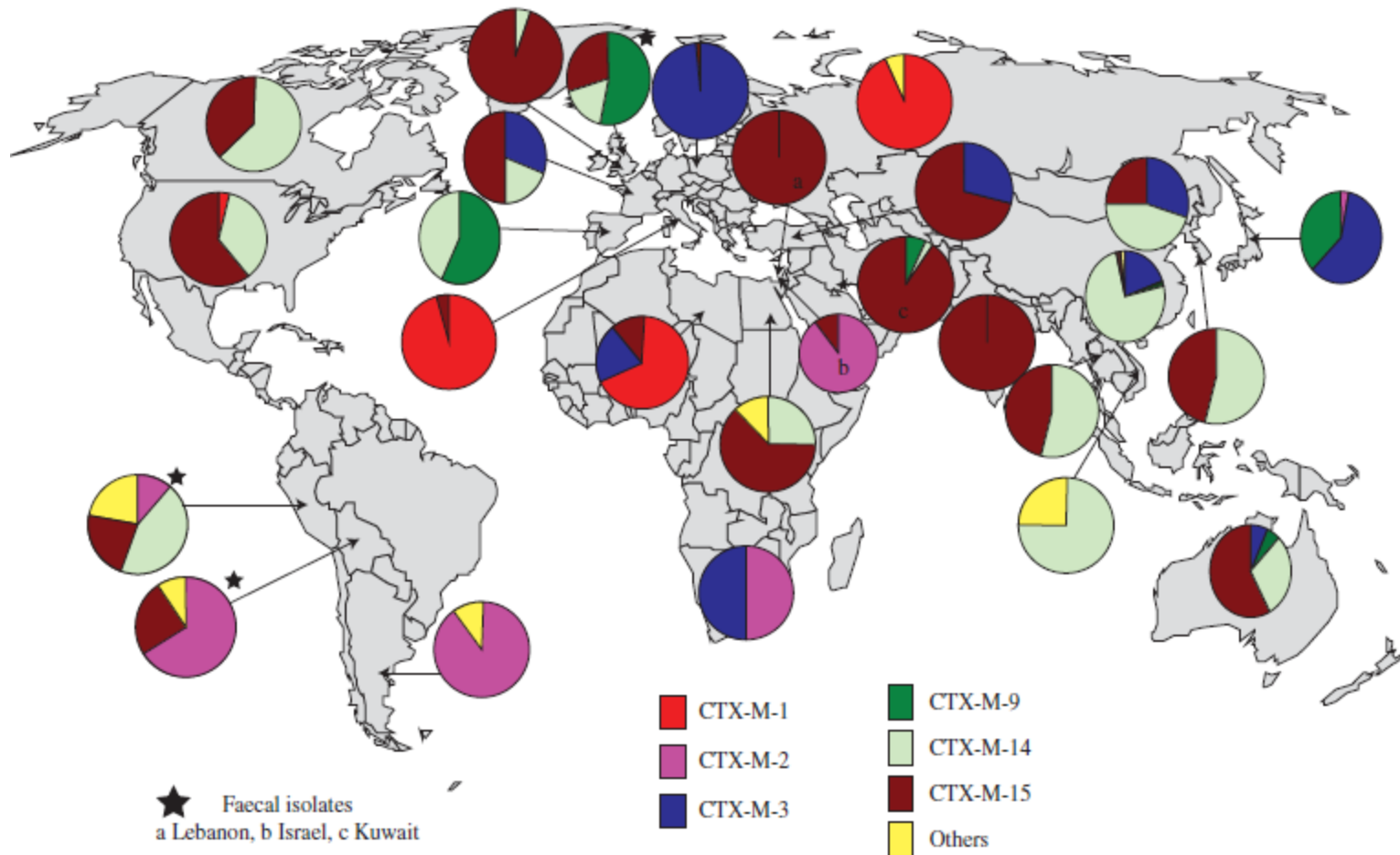
Background:

Resistance to the oxazolidinone antibiotic linezolid is rare (<1% of isolates), but can occur in enterococci and staphylococci. It usually involves mutations (most often G2576T) in chromosomal genes encoding 23S ribosomal RNA, the target of oxazolidinones. This type of resistance can be selected during therapy, but the risk is small unless treatment is prolonged (several weeks). Mutational resistance is not transferable, so infection control to prevent onward transmission of the strain will also prevent spread of the resistance.

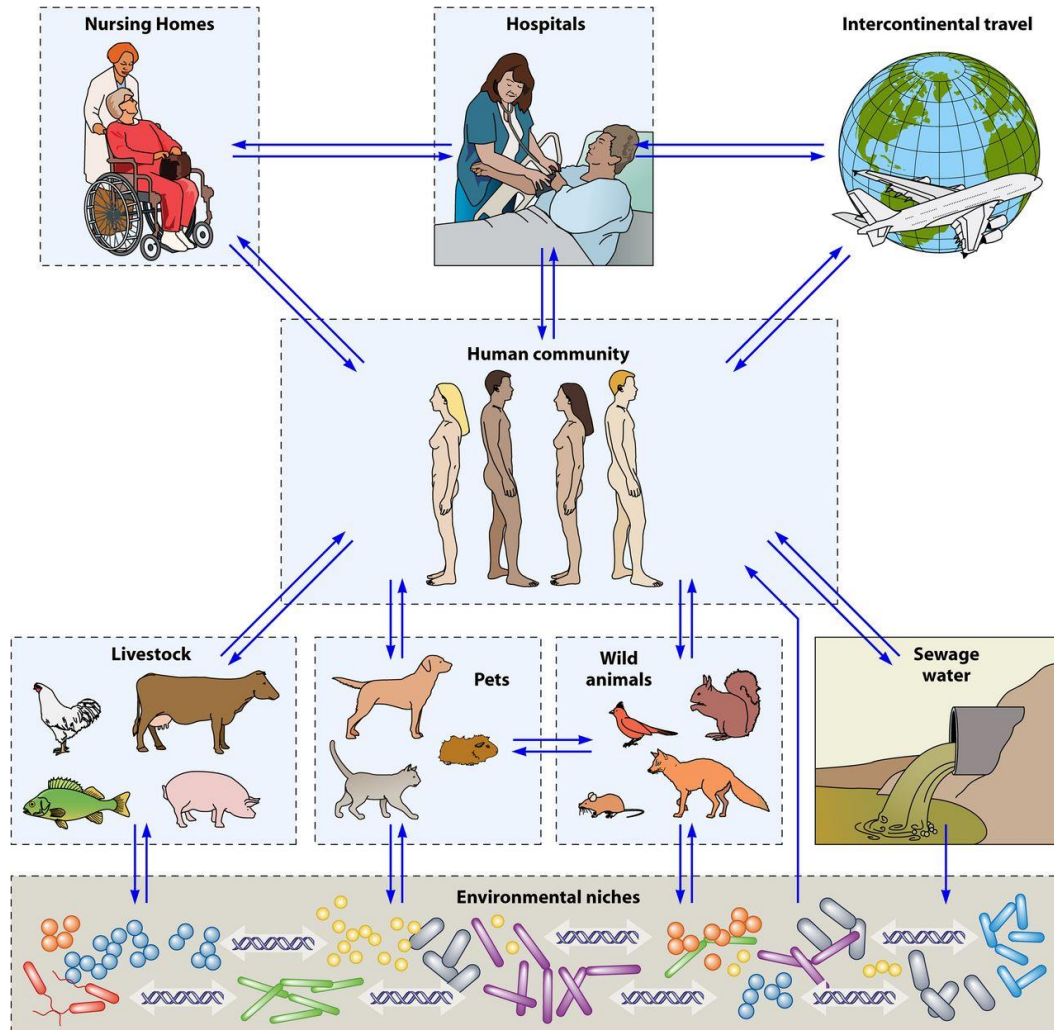
Plasmid-mediated linezolid resistance has also been described, mediated by the *cfr* gene. *cfr* encodes a 23S rRNA methyltransferase, which modifies ribosomal RNA to block binding of linezolid (and clindamycin, chloramphenicol, and dalbavancin). *cfr* has been found mostly in staphylococci (both *S. aureus* and coagulase-negative species) from both humans and animals, with outbreaks in Spain and the USA. There are only three reports of *cfr* in enterococci. The first, from 2010, describes one *E. faecalis* and two *E. faecium* in Madrid; subsequent reports describe single *cfr*-positive *E. faecalis* isolates in Bangkok (Thailand) and Sichuan (China). Prior to the events below, *cfr* had not been found in the UK. *Cfr* has greater public health potential than mutational linezolid resistance since infection control measures may not successfully contain the resistance as the *cfr* gene may 'escape,' transferring among strains, species and genera and establishing reservoirs in skin and gut bacteria.

- Seven *cfr*-positive VRE from 5 patients on a UK renal patient
- High resistance to linezolid (MICs > 8 mg/l)
- Index-patient
 - Admitted to hospital after travel from India
 - Co-colonization with VIM- *P. aeruginosa* and NDM- *E. coli*

Worldwide dissemination of ESBL type CTX-M



Globalization of ESBL-positive Enterobacteriaceae in the community from different reservoirs



Emergence ESBL positive *E. coli* in animals

- Livestock animals and pets
 - Dogs, horses, poultry
- Meat contamination (the Netherlands, 2009)
 - 112 samples (total 262) contaminated by ESBL positive Enterobacteriaceae
 - Chicken (34,0%), beef (32,4%), pork (21,8%)

DeMorgen.be

NIEUWS DE GEDACHTE FINANCIËLE MORGEN PLANET WATCH SPORT

dmgezondheid

Experts slaan alarm over nieuwe superbacterie



La résistance de la bactérie ESBL transmissible aux bactéries humaines

n.c.

Mardi 11 mai 2010

Les bactéries à large spectre productrices de lactamase B, que l'on trouve fréquemment dans les poulets à rôtir, peuvent transmettre leur résistance aux antibiotiques à des bactéries de l'homme, selon les recherches menées par Annemieke Smet dans le cadre de son doctorat à la faculté de médecine vétérinaire de l'Université de Gand. Ces recherches montrent que 60 pc des animaux, dans les entreprises de poulets à rôtir, sont porteurs de la bactérie à large spectre productrice de lactamase B. *E. coli*. Il s'agit de bactéries *E. coli* qui produisent l'enzyme

New superbug resistant to antibiotics and more difficult to tackle than MRSA

Mark Gould and Denis Campbell

vk.nl

de site van de Volkskrant

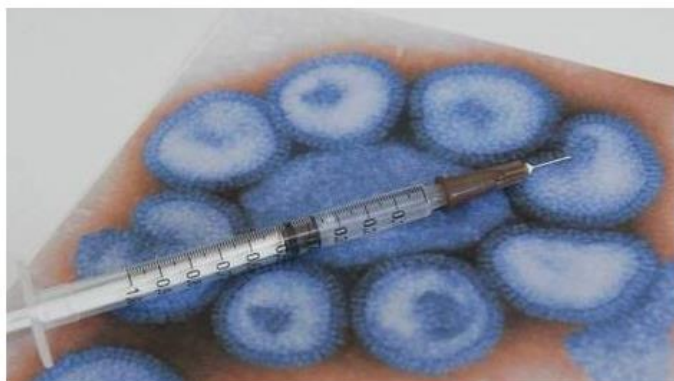
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Superziekteverwekker verspreid

ANP - 17/11/10, 15:49



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STOCKHOLM - De onbehandelbare ziekteverwekker NDM-1 is inmiddels opgedoken in dertien Europese landen. Totaal zijn 77 mensen geïnfecteerd, met name in Groot-Brittannië. Zeven zijn er overleden, meldde het EU-agentschap voor ziektepreventie ECDC woensdag.

De opkomst van de NDM-1 is erg verontrustend, zei Dominique Monnet van de

La "superbactérie" venue d'Inde se répand en Europe

LEMONDE.FR avec Reuters | 17.11.10 | 11h12 • Mis à jour le 17.11.10 | 11h14

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La NDM-1, bactérie particulièrement résistante venue d'Inde et découverte pour la

Mise à jour 21:59

LE FIGARO • fr

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Exemples : Médias, Présidentielle, Auto, Hippisme, Immobilier

Rechercher dans le Fi

Les bactéries ultrarésistantes gagnent du terrain

Mots clés : BACTERIES, GENETIQUE

Par Yves Miserey

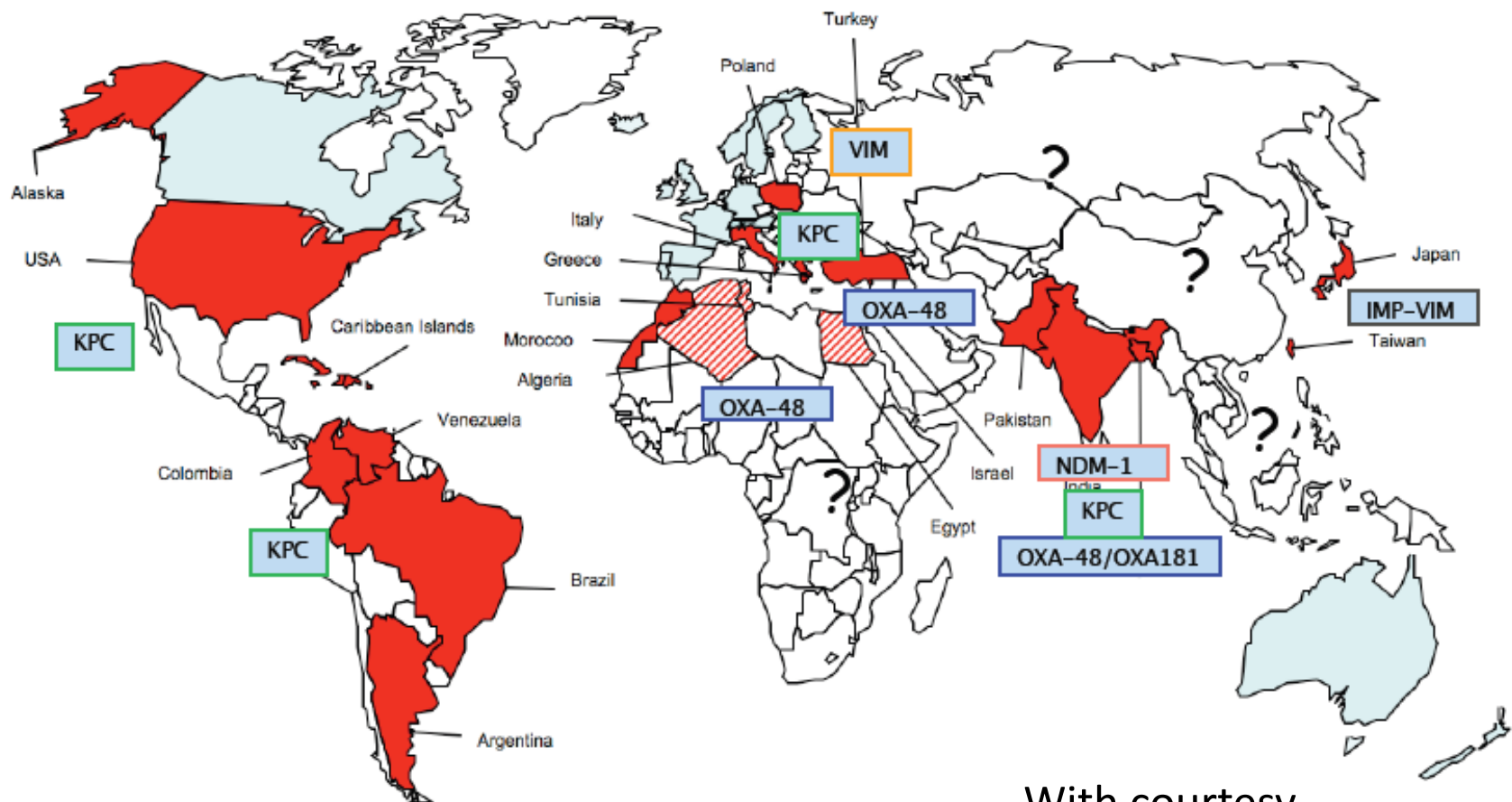
18/01/2011 | Mise à jour : 09:16 Réactions (4)

S'ABONNER AU FIGARO.FR - 8 €/mois

Un Irakien blessé lors d'un attentat à Bagdad et soigné dans un hôpital français était porteur de bactéries dotées du gène de résistance NDM-1.



Carbapenemases- *Enterobacteriaceae* Reservoirs



With courtesy
From P. Nordmann

Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study

Timothy R Walsh, Janis Weeks, David M Livermore, Mark A Toleman



Figure 1: Map of NDM-1-positive samples from New Delhi centre and surrounding areas



Colistin resistance: a major breach in our last line of defence



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See **Articles** page 161

In hospital practice, clinicians have been buoyed by the recent development of new antibiotics active against multidrug resistant Gram-negative bacilli. However, recently approved antibiotics like ceftazidime-avibactam or ceftolozane-tazobactam do not provide activity against all Gram-negative bacilli, with notable gaps in their coverage, including the notorious New Delhi metallo- β -lactamase 1-producing organisms and many strains of carbapenem resistant *Acinetobacter baumannii*. For this reason, the polymyxins (colistin and polymyxin B) remain the last line of defence against many Gram-negative bacilli. Colistin-resistant and even pan-drug-resistant Gram-negative bacilli have already been reported.^{1,2} Typically, colistin resistance

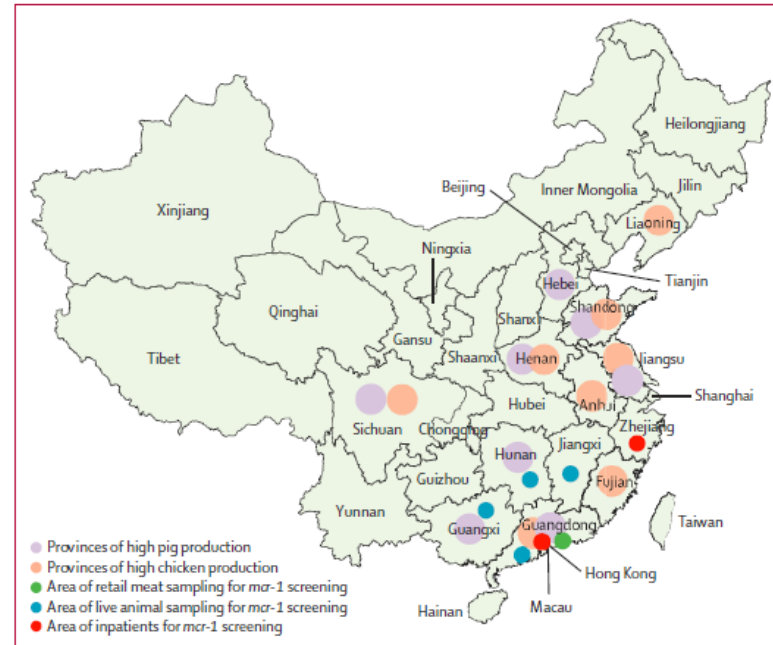


Figure 1: Map of China

mcr-1 Plasmid-mediated gene
encoding phosphoethanolamine
transferase
Modification lipid A

Transferable resistance to colistin: a new but old threat

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In this Leading article, we summarize current knowledge of the occurrence of the first and so far only transferable colistin resistance gene, *mcr-1*. Its location on a conjugative plasmid is likely to have driven its spread into a range of enteric bacteria in humans and animals. Screening studies have identified *mcr-1* in five of the seven continents and retrospective studies in China have identified this gene in *Escherichia coli* originally isolated in the 1980s, while the first European isolate dates back to 2005. Based on the widespread use of colistin in pigs and poultry in several countries and the higher number of *mcr-1*-carrying isolates of animal origin than of human origin, it is tempting to assume that this resistance may have emerged in the animal sector. Whatever its origin, interventions to reduce its further spread will require an integrated global one-health approach, comprising robust antibiotic stewardship to reduce unnecessary colistin use, improved infection prevention, and control and surveillance of colistin usage and resistance in both veterinary and human medicine.

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Schwartz JAC 2016
Liu YY L ncet Infect Dis 2016

Table 1. Isolation of bacteria carrying *mcr-1*

Bacteria	Host	Country	Year(s) of isolation	Reference(s)
<i>E. coli</i>	chicken meat, pork, pigs, humans	China	2011–14	5
<i>K. pneumoniae</i>	human	China	2014	5
Unknown	human microbiome	China	before 2011	8
<i>E. coli</i> , <i>K. pneumoniae</i>	humans	China	2014–15	9,10
<i>E. coli</i>	chicken meat	China	2014	11
Unknown	human microbiome	China	before 2011	12
<i>E. coli</i>	humans	China	2015	13
<i>E. coli</i>	chickens	China	1980–89, 2004, 2006, 2009–14	14
<i>E. aerogenes</i> , <i>E. cloacae</i>	humans	China	2014	15
<i>E. coli</i>	humans	China	2015	16
<i>E. coli</i>	human	China	2015	17
<i>E. coli</i>	dogs, cats	China	2016	17
<i>E. coli</i>	humans	China	2015	18
<i>E. coli</i>	human	Cambodia	2012	19
<i>E. coli</i>	cattle, pig	Japan	2012–13	20
<i>E. coli</i>	humans, pigs	Laos	2012	21
<i>E. coli</i>	pigs, chickens	Malaysia	2013	8
<i>E. coli</i>	chickens, pig, water	Malaysia	2013	22
<i>E. coli</i>	chickens, pig, human, chicken feed, water	Malaysia	2013	23
<i>E. coli</i>	humans	Taiwan	2010, 2012, 2014	24
<i>E. coli</i>	retail meat (beef, chicken, pork)	Taiwan	2012–13, 2015	24
<i>E. coli</i>	humans	Thailand	2012	21
<i>E. coli</i>	pigs and slaughterhouse environment	Vietnam	2014–15	25
<i>E. coli</i>	chicken, pigs	Vietnam	2013–14	26
<i>S. sonnei</i>	human	Vietnam	2008	27
<i>E. coli</i>	calves, piglets	Belgium	2011–12	28
<i>E. coli</i>	pigs	Belgium	2011–12	29
<i>E. coli</i>	human patient, imported chicken meat	Denmark	2012–15	30
<i>E. coli</i>	humans	France	2012	21
<i>Salmonella</i> Derby	sausage	France	2013	31
<i>Salmonella</i> Paratyphi B	food of poultry origin	France	2012	31
<i>Salmonella</i> 1,4,[5],12:–	boot swab from broiler farm	France	2013	31
<i>E. coli</i>	veal calves	France	2005–14	32
<i>E. coli</i>	pigs	France	2011, 2013	33
<i>E. coli</i>	broilers	France	2013–14	33
<i>E. coli</i>	turkeys	France	2014	33
<i>E. coli</i>	pigs	Germany	2010–11	34
<i>E. coli</i>	human	Germany	2014	34
<i>E. coli</i>	humans	Great Britain	2013–14	35
<i>Salmonella</i> 1,4,[5],12:–	humans	Great Britain	2012, 2014–15	35
<i>Salmonella</i> Typhimurium	humans	Great Britain	2015	35
<i>Salmonella</i> Virchow	human	Great Britain	2014	35
<i>Salmonella</i> Paratyphi B	poultry meat, human	Great Britain	2014–15	35
<i>E. coli</i>	pigs	Great Britain	2015	36
<i>Salmonella</i> Typhimurium	pig	Great Britain	2015	36
<i>E. coli</i>	humans	Italy	2013–15	37
<i>E. coli</i>	humans	Italy	2015	38
<i>E. coli</i>	European herring gull	Lithuania	2016	39
<i>E. coli</i>	human	Poland	2015	40
<i>Salmonella</i> Typhimurium	food sample	Portugal	2011	8
<i>Salmonella</i> Typhimurium	retail meat (chicken, beef, pork)	Portugal	2011–12	41
<i>E. coli</i>	turkeys	Spain	2011, 2013–14	42
<i>Salmonella</i> Typhimurium	pigs	Spain	2009–11	42

This study was supported by grants to the German Center of Infection Research (DZIF), and the Zoonoses Network extended-spectrum β -lactamase and fluoroquinolone resistance in Enterobacteriaceae (RESET) Consortium through the German Federal Ministry of Education and Research (BMBF; grant numbers 8000 701-3 [HZI] to TC [T106.001] and CI [01KI1313G]). The study was approved by the ethics committee of the medical faculty of the Justus-Liebig-University of Giessen (AZ: 95/11). All samples were taken as part of standard care procedures. We thank Christina Gerstmann for excellent technical assistance and our collaboration partners from DZIF and RESET for providing the isolates. We declare no competing interests.

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- 3 Paterson DL, Harris PNA. Colistin resistance: a major breach in our last line of defence. *Lancet Infect Dis* 2015; published online Nov 18. [http://dx.doi.org/10.1016/S1473-3099\(15\)00463-6](http://dx.doi.org/10.1016/S1473-3099(15)00463-6).

Colistin resistance gene *mcr-1* harboured on a multidrug resistant plasmid

In *The Lancet Infectious Diseases*, Yi-Yun Liu and colleagues reported, for the first time, plasmid-mediated colistin resistance in *Escherichia coli* isolated from animals, food, and patients in China.¹ These data bring to the fore an as yet unknown facet of colistin resistance and yet again show the effect of antibiotic use in animal farming on human health.^{2,3} We screened a selection of 105 colistin-resistant

that did not include the transposase-encoding *tnpA* gene. pKH-457-3-BE showed 99% similarity (73% query coverage) to plasmid pHX0908 (GenBank access number KM877269) identified in *Salmonella enterica* serotype Typhimurium isolated from chicken stool in China. By contrast with

pHNSHP45, pKH-457-3-BE harboured several resistance-encoding genes to trimethoprim (*dhfrA1*), tetracycline (*tetA*), aminoglycoside (*aadA1*, *aph(6)-Ib* or *strA*, and *aph(3'')-Ib/strB*), and sulphonamide (*sul1*) antibiotics. Phenotypic testing

showed absence of extended-spectrum β -lactamase and carbapenemase production in all *mcr-1* positive strains.

We show a marked presence of *mcr-1* in animal pathogenic bacteria in Europe, an indication that this is already a truly global phenomenon. That *mcr-1* was present in *E coli* circulating in Belgian farm animals during 2011–12 and was harboured on a different plasmid backbone than the one isolated from pigs in China (IncI2) or from imported chicken meat in Denmark (IncX4),^{1,4} indicates a high promiscuity of this gene guided by the adjoining mobile element.



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†Members of the RESET consortium listed in appendix

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- 2 Hasman H, Hammerum A, Hansen F, et al. Detection of *mcr-1* encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, Denmark 2015. *Euro Surveill* 2015; published online Dec 10. <http://dx.doi.org/10.2807/1560-7917.ES.2015.20.49.30085>.
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RAPID COMMUNICATIONS

Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016

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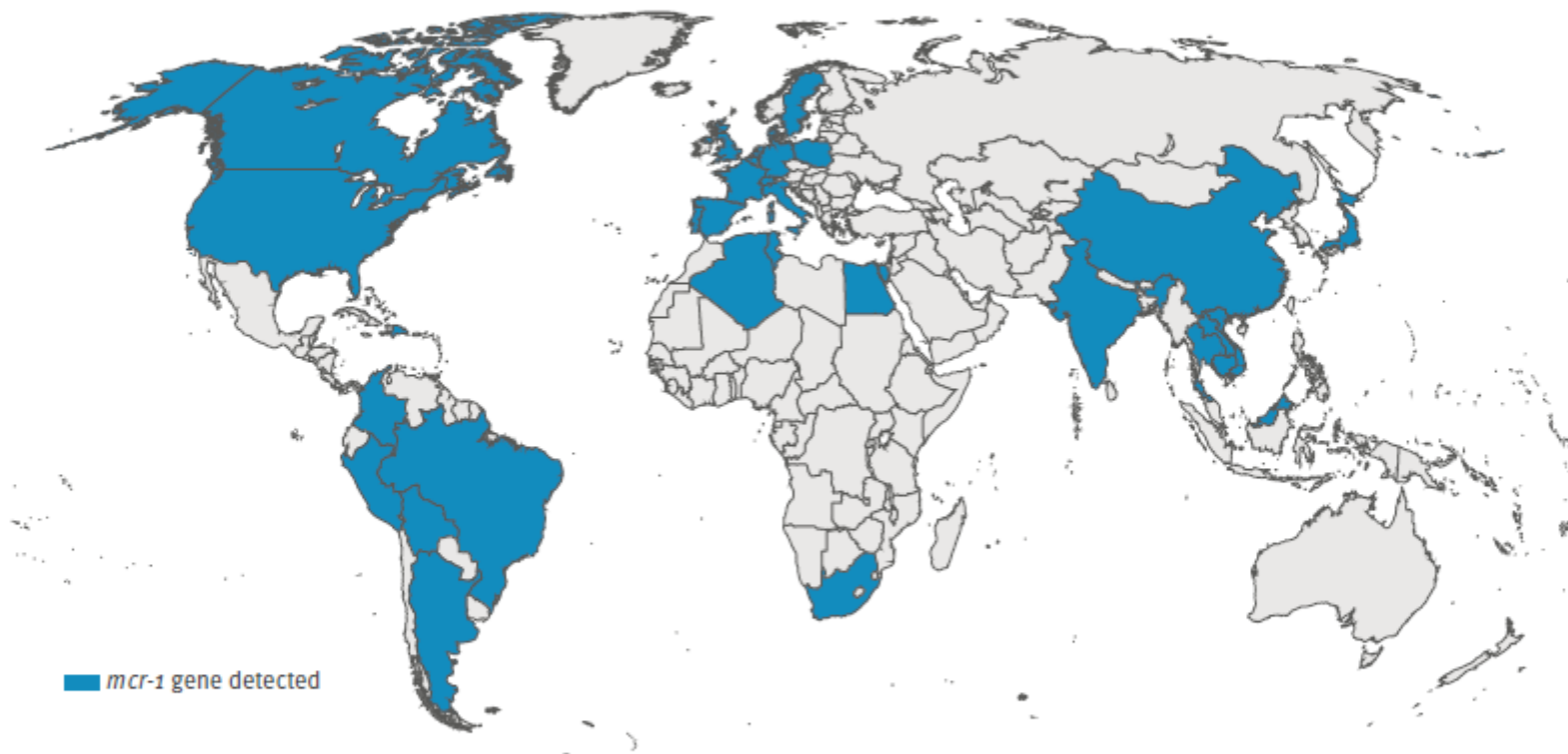
Xavier BB, Lammens C, Ruhel R, Kumar-Singh S, Butaye P, Goossens H, Malhotra-Kumar S. Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016. *Euro Surveill*. 2016;21(27):pii=30280. DOI: <http://dx.doi.org/10.2807/1560-7917.ES.2016.21.27.30280>

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FIGURE 4

Countries (n = 30) reporting presence of *mcr-1* in samples of animal, environmental or human origin (data collected till 27 June 2016)



Adapted from [15]; updated using data from [14,16,17,25-27].



Resistance is no more limited to bacteria

RAPID COMMUNICATIONS

Azole-resistant *Aspergillus fumigatus* due to TR46/Y121F/T289A mutation emerging in Belgium, July 2012

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RESEARCH ARTICLES

Azole-resistant *Aspergillus fumigatus* in the environment of northern Italy, May 2011 to June 2012

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