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

- Transposon
- Intégron
- Cassette
- Plasmid

Virulence factors

- Co-resistance; co-expression; co-selection
Horizontal transfers of resistance genes

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

- Pseudomonas
- Enterobacteriaceae
- Vibrio cholerae
- Campylobacter
- Staphylococci
- Enterococci
- Staphylococci
- Enterococci
- Streptococci
- Pneumococci

Tenover F, CID 2001; 33: S108-115
Transmission and spread of antimicrobial resistance

• Horizontal and vertical transmission
  – Vertical transmission by clonal expansion
  – Horizontal transmission: plasmid mediated conjugation
Transmission and spread of antimicrobial resistance

- **Microscopic and macroscopic transmission**

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**Cytoplasmic bridge**

**Air bridge**

Sex pilus

2 μm
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

\[
\text{mec gene complex} \quad \text{ccr gene complex}
\]

Ito t. et al. Antimicrob Agents Chemother 2012;4997
Staphylococcal Cassette Chromosome Chromosome mec

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

<table>
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<tr>
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Staphylococcal Cassette Chromosome mec

Classification

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

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<td>B3</td>
<td>E</td>
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</table>
MRSA transmission between humans and pets

• Transmission within households, veterinary hospitals and farms
  – Dogs, cats but also rabbits, parrots, ... horses

– HA-MRSA
  • ST22-SCCmec IV, ST8-SCCmec II
  • Transmission of nurse to her baby and dog

– PVL- positive CA-MRSA
  • ST80-SCCmec IV, USA300 ST8-SCCmec IV
  • Familial outbreaks with recurrent infection

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
MRSA and livestock animals

- **France**
  - Molecular analysis
  - 44 S. aureus from healthy pig farmers over 7 departments
  - 14 S. aureus from swine infections
  - 5 MRSA
  - 16 ST including ST9 and ST398

- **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 *Smal* 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 SCC\textit{mec} 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

Denis o. et al. EID 2009
### MRSA carriage among veterinarians in Belgium and Denmark

<table>
<thead>
<tr>
<th>Exposure variable</th>
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<th>Denmark</th>
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<th>P value*</th>
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<tr>
<td>Total MRSA</td>
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<td>2 (1.4)</td>
<td>7.5 (1.7–68.6)</td>
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<td>(n=97)</td>
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<td>6.7 (1.5–62.4)</td>
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<td>(n=41)</td>
<td>(n=46)</td>
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<td>MRSA ST398</td>
<td>0 (0)</td>
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<tr>
<td>MRSA non-ST398</td>
<td>1 (2.4)</td>
<td>0 (0)</td>
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<tr>
<td>Total MRSA</td>
<td>1 (2.4)</td>
<td>0 (0)</td>
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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, Willem van Wamel, Stien Vandendriessche, Jesper Larsen, Olivier Denis, Cristina Garcia-Graells, Ann-Catrin Uhlemann, Franklin D. Lowy, Robert Skov, and Jodi A. Lindsay

- Human clade associated with bacteriophage ϕ3
  - Immune Evasion Cluste
  - Absence of tetM gene encoding tetracycline resistance
  - MSSA
- LA-MRSA
  - MRSA
  - tetM gene
  - Bacteriophage ϕ7
Lifestock-associated CC398 MRSA

- **Multi-resistant**
  - To antibiotics
  - High diversity of resistance genes
    - 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\textsubscript{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

Kadlec K et al. AAC 2010
Methicillin-susceptible *Staphylococcus aureus* ST398-t571 harbouring the macrolide–lincosamide–streptogramin B resistance gene *erm(T)* in Belgian hospitals

**Stien Vandendriessche**¹,²*, **Kristina Kadlec**³, **Stefan Schwarz**³ and **Olivier Denis**¹

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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 Apal 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 (‘ML Sb’) 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


- 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 >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
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
cfr-Positive MRSA ST398

- LA-MRSA ST398
- Resistance to chloramphenicol and clindamycin
- Linezolid susceptible?
- Not detected by disk diffusion using CLSI guidelines
- MIC to linzolid = 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

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Paridaens H et al. submitted
Vanderhaegen W et al. JAC 2013
Peeters LEJ et al. Vet Microbiol 2015
Angeles Argudin M et al Res Vet Science 2015
• 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

Hawkey PM et al. JAC 2009 suppl 1; i3
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%)
New superbug resistant to antibiotics and more difficult to tackle than MRSA

Mark Gould and Denis Campbell

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

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

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
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

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. Typically, colistin resistance

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

Transferable resistance to colistin: a new but old threat

Stefan Schwarz1* and Alan P. Johnson2

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*Corresponding author. Tel: +49-5034-871-241; Fax: +49-5034-871-143; E-mail: stefan.schwarz@fli.bund.de

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.
<table>
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<tr>
<th>Bacteria</th>
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<td>21</td>
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<tr>
<td>Salmonella Derby</td>
<td>sausage</td>
<td>France</td>
<td>2013</td>
<td>31</td>
</tr>
<tr>
<td>Salmonella Paratyphi B</td>
<td>food of poultry origin</td>
<td>France</td>
<td>2012</td>
<td>31</td>
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<tr>
<td>Salmonella 1,4,[5],12:−</td>
<td>boot swab from broiler farm</td>
<td>France</td>
<td>2013</td>
<td>31</td>
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<tr>
<td>E. coli</td>
<td>veal calves</td>
<td>France</td>
<td>2005 – 14</td>
<td>32</td>
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<td>E. coli</td>
<td>pigs</td>
<td>France</td>
<td>2011, 2013</td>
<td>33</td>
</tr>
<tr>
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<td>France</td>
<td>2013 – 14</td>
<td>33</td>
</tr>
<tr>
<td>E. coli</td>
<td>turkeys</td>
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<td>2014</td>
<td>33</td>
</tr>
<tr>
<td>E. coli</td>
<td>pigs</td>
<td>Germany</td>
<td>2010 - 11</td>
<td>34</td>
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<tr>
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<td>human</td>
<td>Germany</td>
<td>2014</td>
<td>34</td>
</tr>
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<td>E. coli</td>
<td>humans</td>
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<td>2013 – 14</td>
<td>35</td>
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<tr>
<td>Salmonella Typhimurium 1,4,[5],12:−</td>
<td>humans</td>
<td>Great Britain</td>
<td>2012, 2014 – 15</td>
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<td>Great Britain</td>
<td>2015</td>
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<td>Salmonella Virchow</td>
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<td>2014</td>
<td>35</td>
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<tr>
<td>Salmonella Paratyphi B</td>
<td>poultry meat, human</td>
<td>Great Britain</td>
<td>2014 – 15</td>
<td>35</td>
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<tr>
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<td>Great Britain</td>
<td>2015</td>
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<td>Salmonella Typhimurium</td>
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<td>2015</td>
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<td>Italy</td>
<td>2015</td>
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<td>E. coli</td>
<td>European herring gull</td>
<td>Lithuania</td>
<td>2016</td>
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<td>Poland</td>
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<td>Salmonella Typhimurium</td>
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<td>Spain</td>
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<td>42</td>
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<td>Salmonella Typhimurium</td>
<td>pigs</td>
<td>Spain</td>
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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.²³ 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 pHXY0908 (GenBank access number KMB77269) 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 (dfrA1), tetracycline (tetA), aminoglycoside (aadA1, aph(6)-Id or sla, and andaph(3")-lb/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),¹⁴ indicates a high promiscuity of this gene guided by the adjoining mobile element.
Rapid Communications


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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 [01K1313G]). 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 that did not include the transposase-encoding trnA gene. pKH-457-3-BE showed 99% similarity (73% query coverage) to plasmid pHXY0908 (GenBank access number KM877269) identified in Salmonella enterica serotype Typhimurium isolated from chicken stool in China. By contrast with pHXY0908, pKH-457-3-BE harboured
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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- W. Vanderhaeghen