

# Nachweis der Übertragung und Veränderung eines Carbapenemase- kodierenden Plasmids im Tiermodell Huhn

Sead Hadziabdic, Jennie Fischer, Burkhard Malorny, Istvan Szabo

# β-Lactam-Antibiotika

- **Meist verwendete Antibiotika**

- 50% Penicilline und Cephalosporine

- **Hemmung der Peptidoglycansynthese**

- Die Bindung an unterschiedlichen PBPs

- **Beta-Lactamase-Inhibitoren**

- Clavulansäure, Tazobactam, Sulbactam

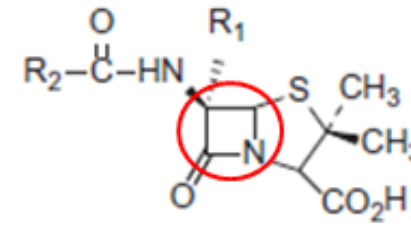
- **Die WHO CIA Liste**

- Sechste Revision (2018)
- Zwei Kriterien (C1 und C2)
- Drei Kategorien:
  - Critically important
  - Highly important
  - Important antimicrobials

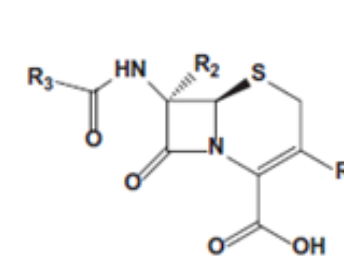
(C1 und C2)

(C1 oder C2)

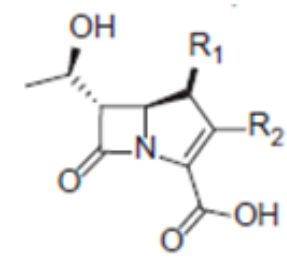
(keine Kriterien erfüllt)



Penicillins



Cephalosporins



Carbapenems

Übernommen von Bush *et al.* (2018)

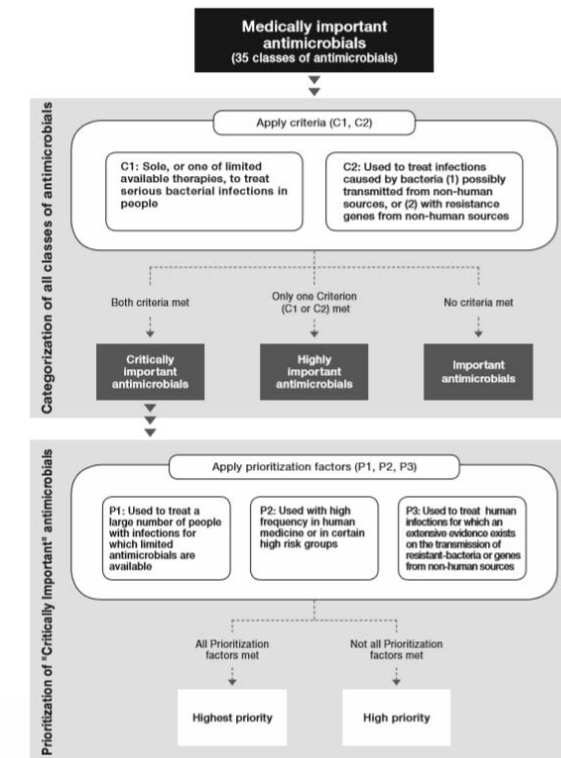
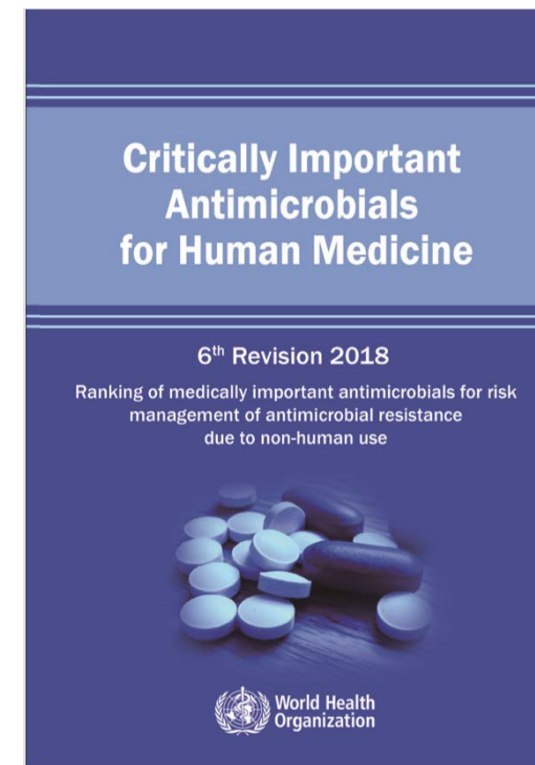
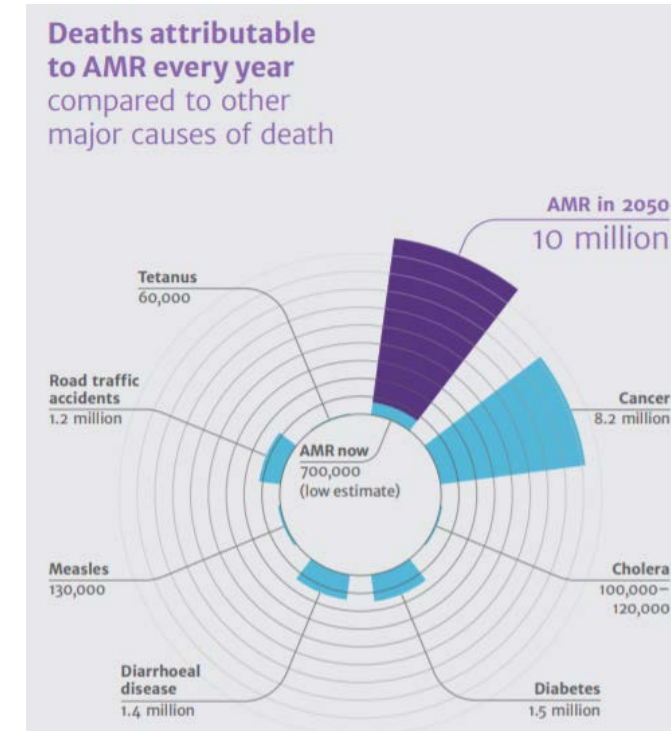


Figure 1. Flow chart of application of criteria and prioritization factors to medically important antimicrobials

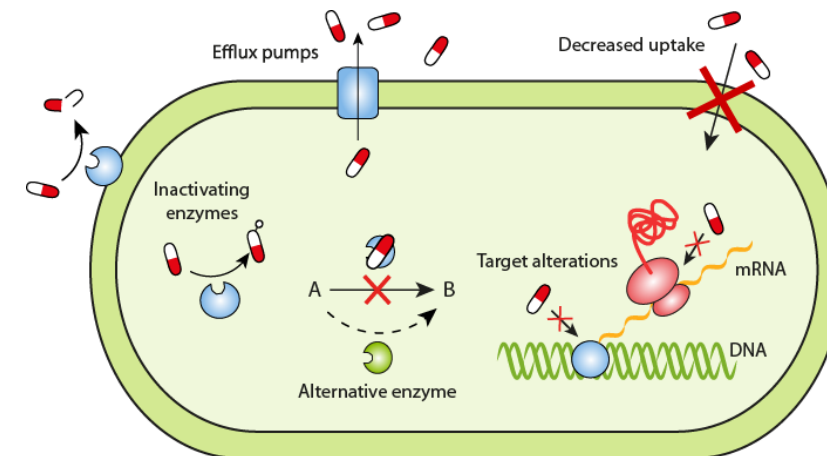
[https://www.who.int/foodsafety/areas\\_work/antimicrobial-resistance/cia/en/](https://www.who.int/foodsafety/areas_work/antimicrobial-resistance/cia/en/)

# Antimikrobielle Resistenz (AMR)

- Superkeime
- UN-Vollversammlung (2016)
- O'Neill Report
  
- **Intrinsische Resistenz**
  - Beringianischer Dauerfrost (D'Costa *et al.* 2011)
  
- **Erworbene Resistenz**
  - Missbrauch von Antibiotika
  - Wachstumsförderung (Verordnung (EG) Nr. 1831/2003)
  
- **Die Mechanismen der Resistenz**
  - Blocken, Pumpen, Spalten und Verwandeln
  
- **Verbreitung der Resistenzen**
  - Vertikal
  - Horizontal (MGEs)



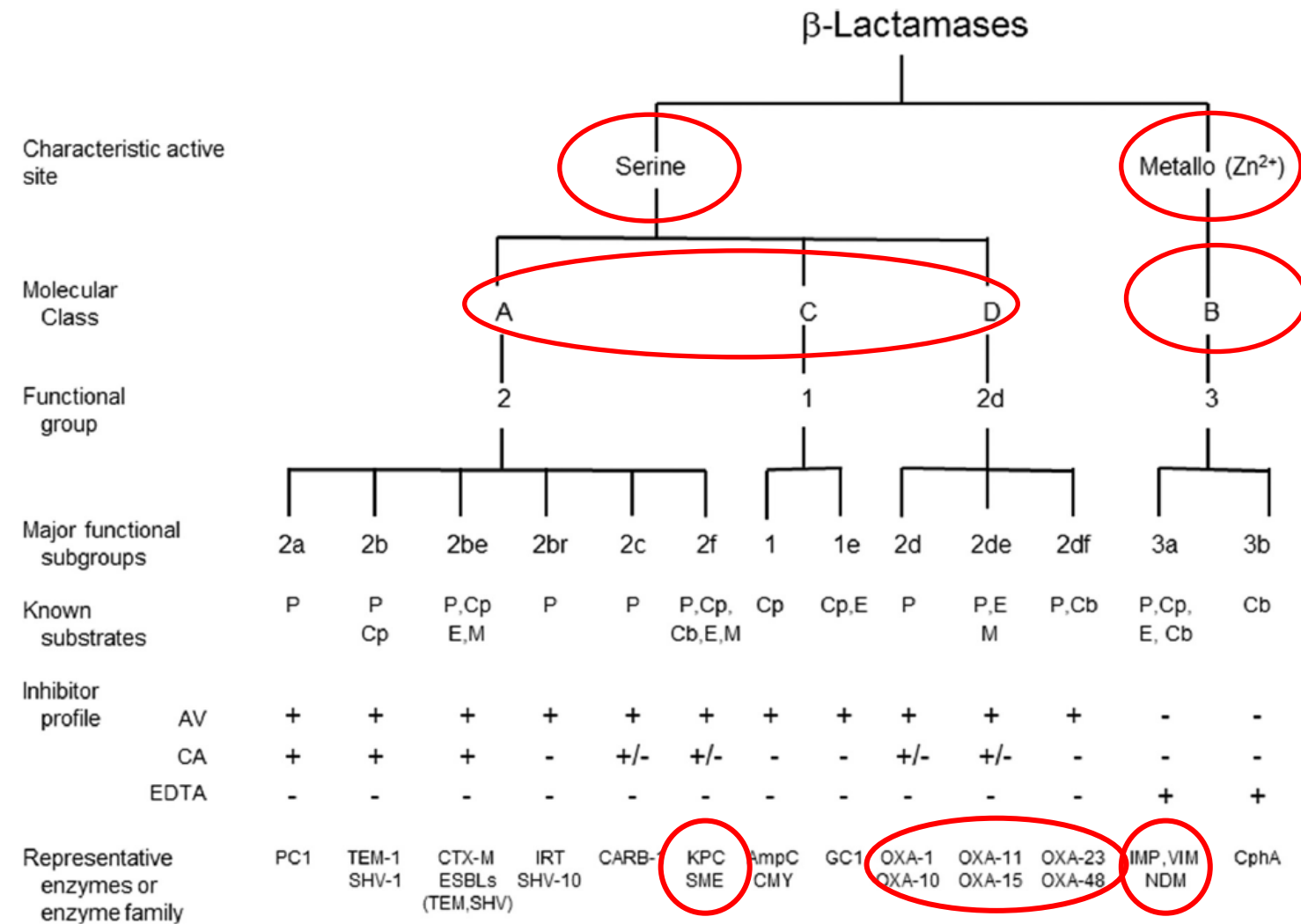
Review on Antimicrobial Resistance. Antimicrobial Resistance: Tackling a Crisis for the Health and Wealth of Nations. 2014.



<https://www.reactgroup.org/toolbox/understand/antibiotic-resistance/resistance-mechanisms-in-bacteria/>

# β-Lactamasen

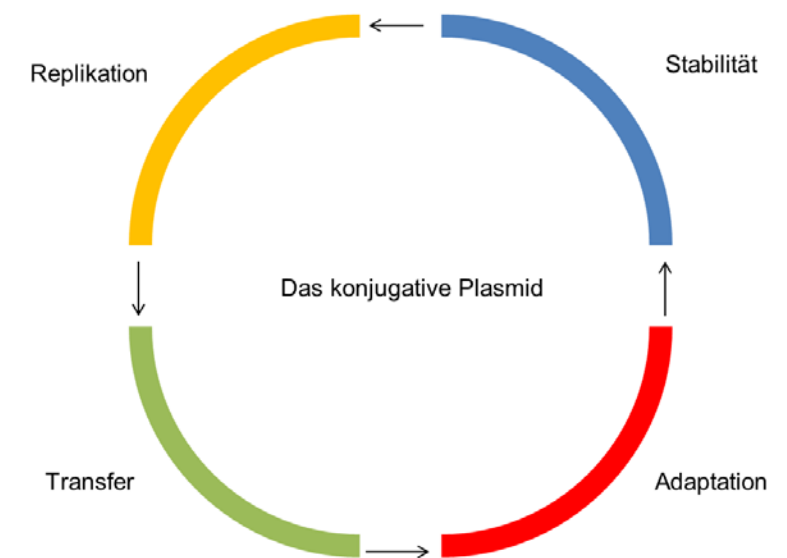
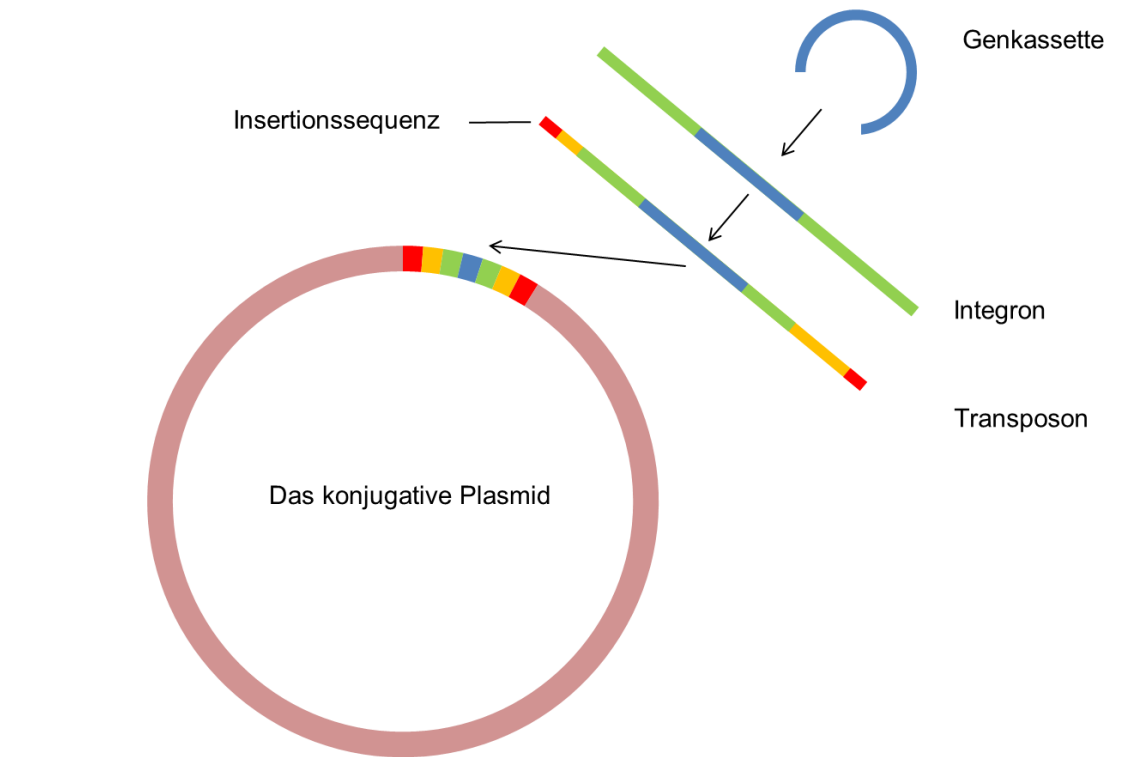
- Zielstruktur ist der **β-Lactam-Ring**
- Meist bekannte Enzyme
  - > 1000 Enzyme
  - Kommensale und pathogene Bakterien
- Wichtigste **β-Lactamasen** sind die **Carbapenemasen**
- Weltweit häufigsten Carbapenemasen
  - **IMP, KPC, NDM, OXA und VIM**  
(*bla*<sub>IMP</sub>, *bla*<sub>KPC</sub>, *bla*<sub>NDM</sub>, *bla*<sub>OXA</sub> und *bla*<sub>VIM</sub>)
- Antibiotika-Einsatz
  - Humanmedizin: höhere Prävalenz
  - Veterinärmedizin: sporadisch (dominant in Asien)



Übernommen von Bush *et al.* (2018)

# Mobile genetische Elemente (MGE)

- Interzelluläre und intrazelluläre MGE
- **Genkassetten**
- **Integrans**
- **Transposons**
- **Plasmide**
  - Verbreitung der Resistenz
  - Konjugative und nicht konjugative Plasmide
  - Kleine und Megaplasamide
  - Antibiotika- und Schwermetallresistenz
  - Viele Gene haben eine unbekannte Funktion
- **Inkompatibilitätsgruppen (Inc)**
  - 28 Inc Gruppen in Enterobakterien
  - IncF, IncI, **IncA/C** und IncH wichtig für AMR

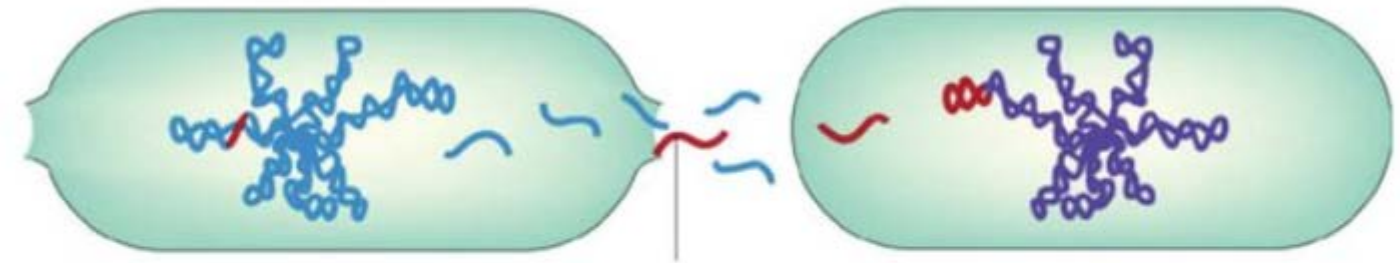


Adaptiert von Norman *et al.* (2009)

# Die Mechanismen des HGT

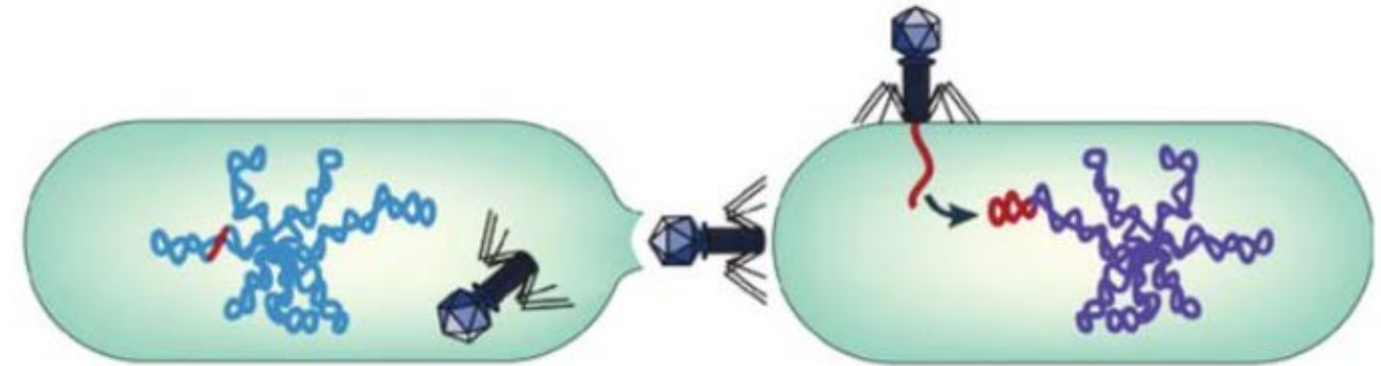
- **Transformation**

- Aufnahme der freien DNA



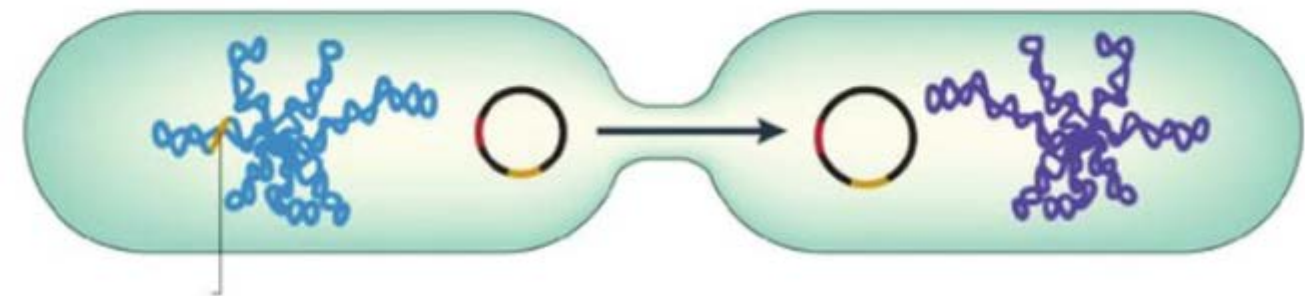
- **Transduktion**

- Viren der Bakterien oder sog. Bakteriophagen



- **Konjugation**

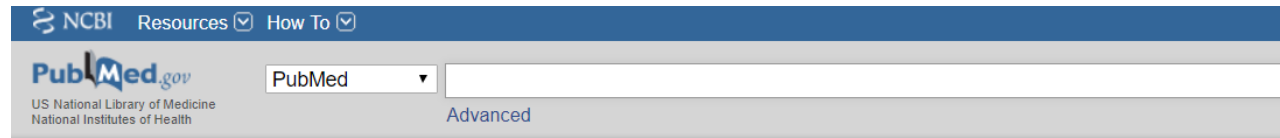
- Wichtig für den Austausch der Plasmide
- Physischer Kontakt zwischen **Donor-** und **Rezipientzelle** und die Entstehung der **Transkonjuganten**
- Zwischen unterschiedlichen Bakterien



Übernommen von Furuya und Lowy, 2006

# Carbapenemase-produzierende Salmonellen bei Tieren (DE)

- **NDM-1 *S. Corvallis*** (Wildvogel)



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*J Antimicrob Chemother.* 2013 Dec;68(12):2954-6. doi: 10.1093/jac/dkt260. Epub 2013 Jun 30.

## NDM-1 carbapenemase-producing *Salmonella enterica* subsp. *enterica* serovar *Corvallis* isolated from a wild bird in Germany.

Fischer J<sup>1</sup>, Schmogger S, Jahn S, Helmuth R, Guerra B.

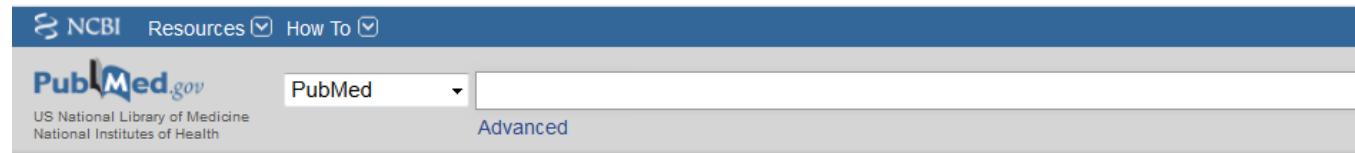
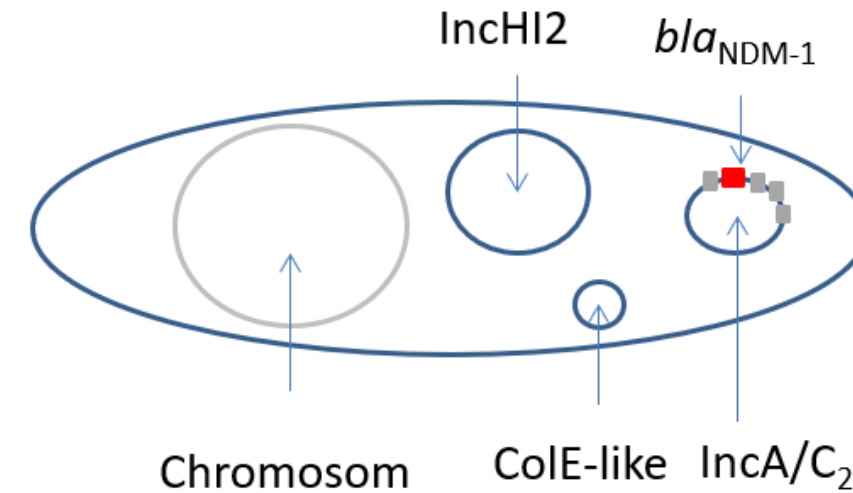
### Author information

<sup>1</sup> Federal Institute for Risk Assessment, BfR, Department for Biological Safety, Max-Dohrn Strasse 8-10, D-10589 Berlin, Germany.

**KEYWORDS:** antimicrobial resistance; black kite; carbapenems; plasmids

PMID: 23818284 DOI: [10.1093/jac/dkt260](https://doi.org/10.1093/jac/dkt260)

[Indexed for MEDLINE]



Format: Abstract Send to ▾

*Genome Announc.* 2018 Jun 28;6(26). pii: e00593-18. doi: 10.1128/genomeA.00593-18.

## Complete Genome Sequence of an Avian Native NDM-1-Producing *Salmonella enterica* subsp. *enterica* Serovar *Corvallis* Strain.

Hadziabdic S<sup>1</sup>, Borowiak M<sup>1</sup>, Bloch A<sup>1</sup>, Malorny B<sup>1</sup>, Szabo J<sup>1</sup>, Guerra B<sup>2</sup>, Kaesbohrer A<sup>1</sup>, Fischer J<sup>3</sup>.

### Author information

<sup>1</sup> Department for Biological Safety, German Federal Institute for Risk Assessment (BfR), Berlin, Germany.

<sup>2</sup> European Food Safety Authority (EFSA), Parma, Italy.

<sup>3</sup> Department for Biological Safety, German Federal Institute for Risk Assessment (BfR), Berlin, Germany Jennie.Fischer@bfr.bund.de.

## Andere Carbapenemase-produzierende Salmonellen

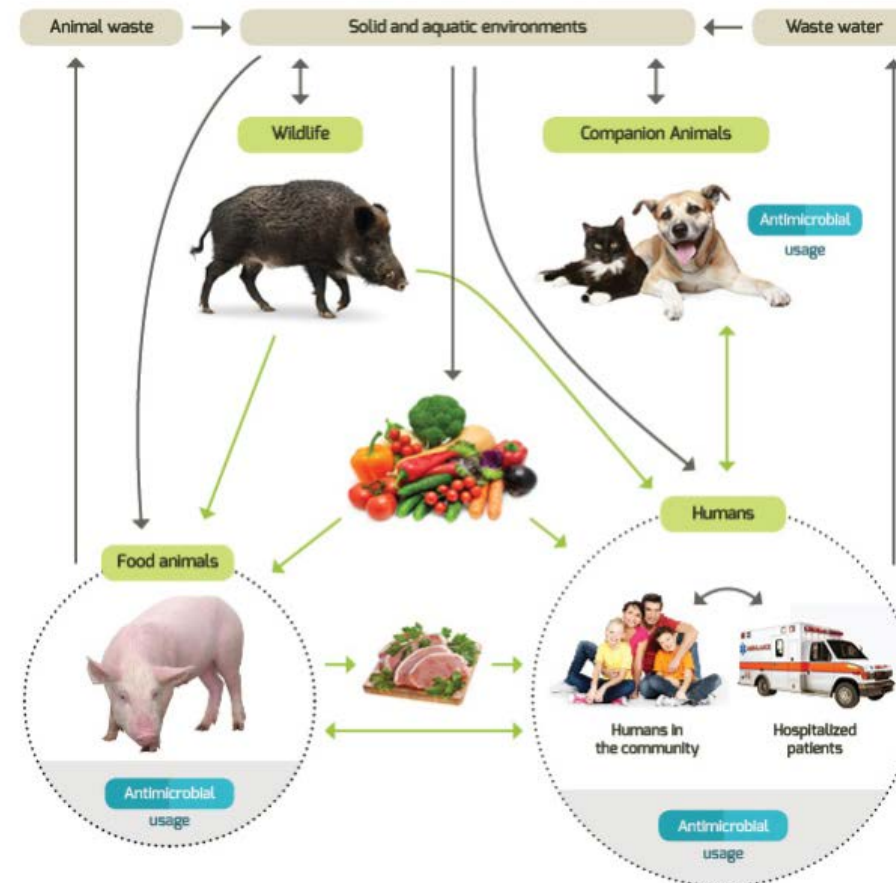
- **VIM-1 *S. Infantis*** (Schwein, Geflügel)
- **VIM-1 *S. Goldcoast*** (Schwein)

# Das EFFORT Projekt

The screenshot shows the EFFORT project website. At the top, it says 'EFFORT AGAINST ANTIMICROBIAL RESISTANCE' and 'Co-funded by the European Union'. The navigation menu includes 'The Project', 'Activities', 'Participants', 'News & Events', 'Publications', and 'Final Conference'. There is a search bar and a language selection dropdown. The main content area features a large image of a DNA helix and a microorganism. Below the image, the text reads 'EFFORT against antimicrobial resistance'. To the left, there are sections for 'NEWS' (Project Final Report and Deliverables) and 'EVENTS' (EFFORT International Conference: How to control antimicrobial resistance in the food chain: Thank you!).

## Activities

The EFFORT project will investigate the epidemiology and ecology of antimicrobial resistance in food-producing animals, the (farm) environment, and food of animal origin as well as companion animals and wildlife to quantify the exposure of humans to AMR through these different exposure pathways. Studies on transmission to humans will be established for some high-risk occupationally exposed populations. The novel understanding will be used to develop and evaluate intervention strategies aimed at managing the public health and animal health risks related to antimicrobial resistance.



- Internationales Projekt
  - 20 Teilnehmer aus 10 Ländern
  - Budget ca. 10-12 Millionen Euro
  - Geendet in November 2018

<http://www.effort-against-amr.eu/>

## WP2

Molecular approaches for determining the molecular ecology and epidemiology of antimicrobial resistance genes

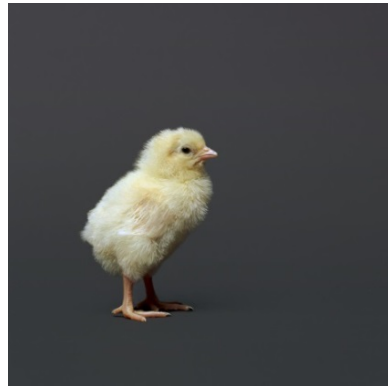
## WP3

Ecology and transfer of resistance mechanisms





# In vivo Modelle für die Untersuchung des HGT



Sandor Somkuti/PIXELIO

Walter Eberl/PIXELIO

- **Guillot *et al.***
- **Hart *et al.***
  - Resistenzplasmid (↑ Tetrazykline)
- **Marosevic *et al.***
  - *erm*(B)-tragenden Plasmid (↑ und ↓)
- **Avrain *et al.***
  - *tet*(O)-tragenden Plasmid

- **Nijsten *et al.***
  - Resistenzplasmid (↑ Lincomycin)
- **Dahl *et al.***
  - Glykopeptid-resistenz
- **Jacobsen *et al.***
  - *Lactobacillus plantarum*
    - ↓
  - *Enterococcus faecalis*

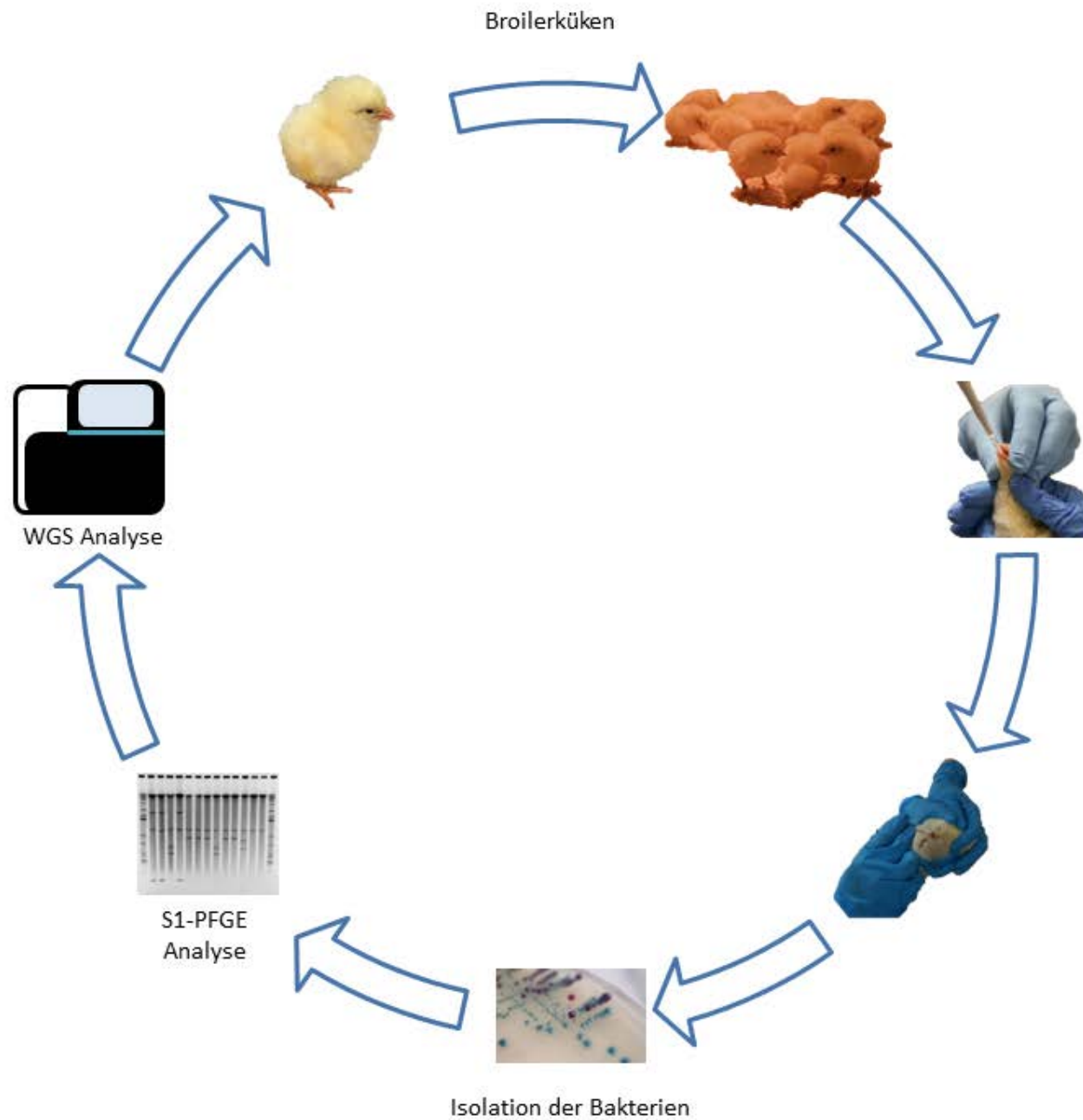
- **Akhtar *et al.***
  - *tet*(M)-tragenden Plasmid
- **Petridis *et al.***
  - Chloramphenicol-resistenz
- **Poole *et al.***
  - *S. Newport*
    - ↓
  - *E. coli*

# Tierversuche am Modell Huhn

- **Szenario:** Simulation des Eintrags eines NDM-1-produzierenden *S. Corvallis* Stammes in einer Broilerherde ohne Antibiotika-Einsatz
- **Ziel:** Das Verständnis der möglichen Konsequenzen
- LAGESO Genehmigung für die Tierversuche (0308/15)
- Tierversuche dauerten je 4 Wochen
- *Per os* Inokulation des Donor-Stammes ( $\sim 10^7$  CFU/Tier)
- Individuelle Beprobung der Tiere
- Tägliche Kontrolle des Gesundheitsstatus
- Ohne erhöhte Mortalitätsrate und Krankheitsanzeichen im Laufe des Tierversuchs

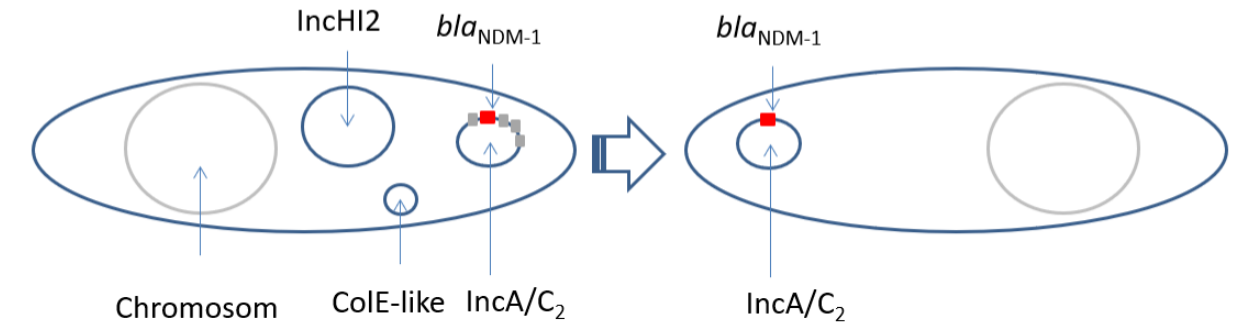


# Tierversuche am Modell Huhn



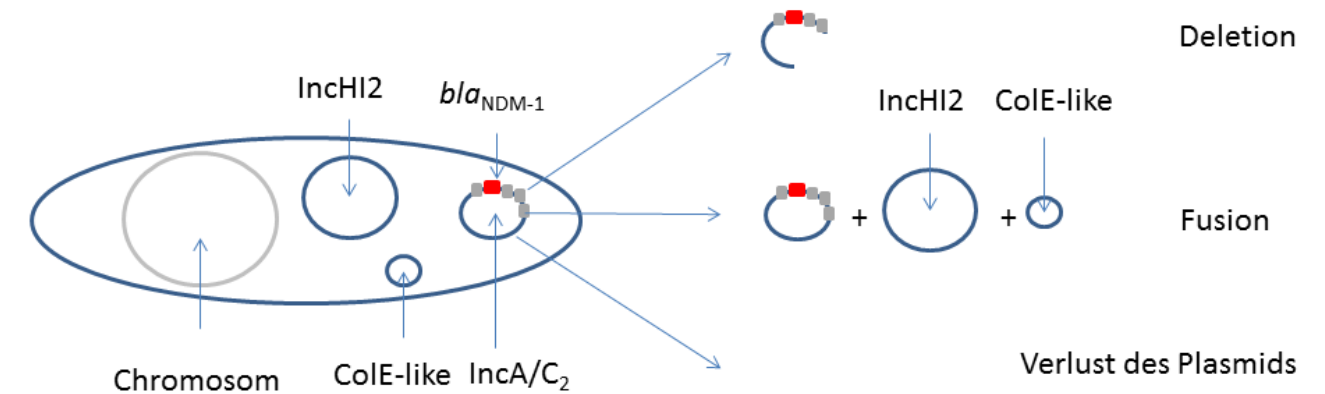
## ○ Fragestellung 1

- *In vivo* Übertragung des  $bla_{NDM-1}$ -tragenden IncA/C<sub>2</sub> Plasmids

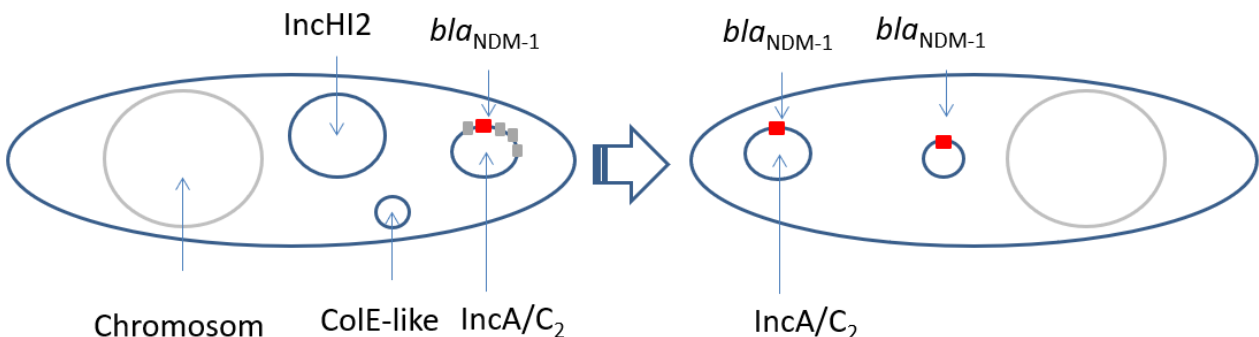


## ○ Fragestellung 2

- Strukturänderung des  $bla_{NDM-1}$ -tragenden IncA/C<sub>2</sub> Plasmids



# In vivo Übertragung des $bla_{\text{NDM-1}}$ -tragenden IncA/C<sub>2</sub> Plasmids

- Ab 2. bis 3. Tag p.i Isolierung von Transkonjuganten
  - *E. coli* (ST-117, ST-156, ST-2040, ST-2485)
  - *K. pneumoniae* (ST-1106)
- Transkonjuganten konnten bis Ende des Tierversuchs nachgewiesen werden
- Transposition des  $bla_{\text{NDM-1}}$  Genes
 
- Das  $bla_{\text{NDM-1}}$ -tragende IncA/C<sub>2</sub> Plasmid blieb stabil in den Transkonjuganten

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US National Library of Medicine  
National Institutes of Health

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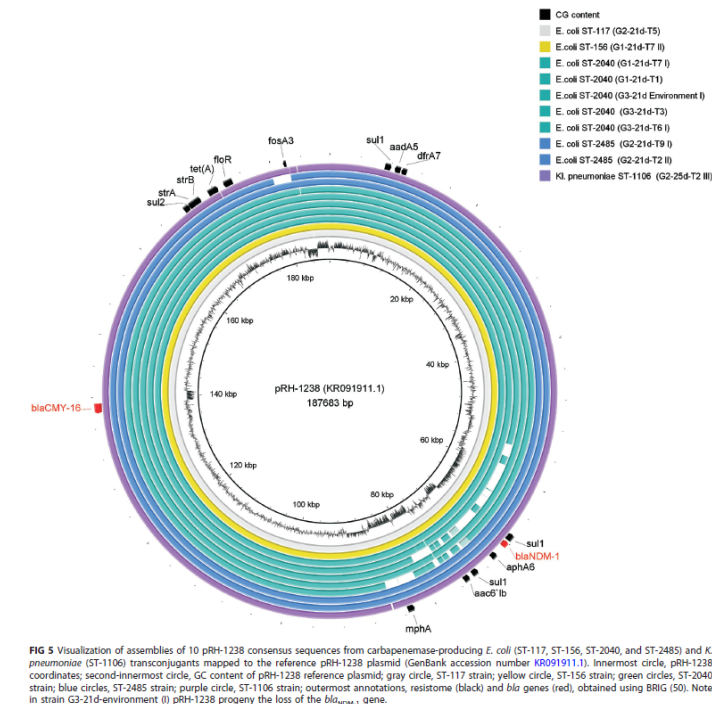
Antimicrob Agents Chemother. 2018 Mar 27;62(4). pii: e02128-17. doi: 10.1128/AAC.02128-17. Print 2018 Apr.

**In Vivo Transfer and Microevolution of Avian Native IncA/C<sub>2</sub> $bla_{\text{NDM-1}}$ -Carrying Plasmid pRH-1238 during a Broiler Chicken Infection Study.**

Hadziabdic S<sup>1</sup>, Fischer J<sup>1</sup>, Malorny B<sup>1</sup>, Borowiak M<sup>1</sup>, Guerra B<sup>1</sup>, Kaesbohrer A<sup>1</sup>, Gonzalez-Zorn B<sup>2</sup>, Szabo I<sup>3</sup>.

**Author information**

- 1 German Federal Institute for Risk Assessment (BfR), Department for Biological Safety, Berlin, Germany.
- 2 Departamento de Sanidad Animal and Centro de Vigilancia Sanitaria Veterinaria, Facultad de Veterinaria, Universidad Complutense de Madrid, Madrid, Spain.
- 3 German Federal Institute for Risk Assessment (BfR), Department for Biological Safety, Berlin, Germany istvan.szabo@bfr.bund.de.

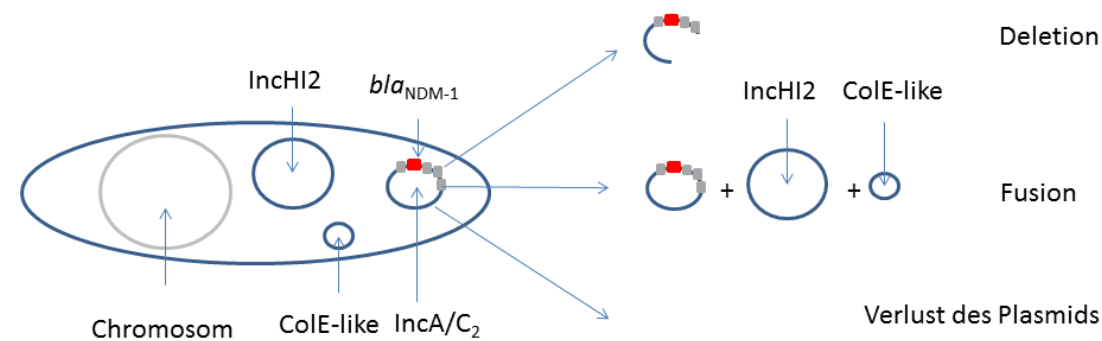


# Die Strukturänderung des *bla*<sub>NDM-1</sub>-tragenden IncA/C<sub>2</sub> Plasmids

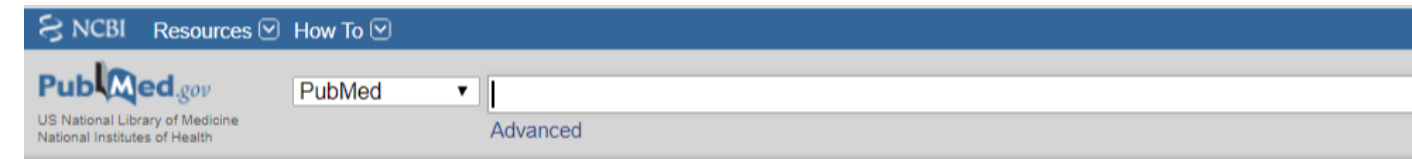
Das *bla*<sub>NDM-1</sub>-tragende IncA/C<sub>2</sub> Plasmid blieb stabil im *S. Corvallis* Donor-Stamm und konnte bis Ende des Tierversuchs nachgewiesen werden

## Die Strukturänderung

- Kleinere (~10 kb) Deletion
- Größere (~70 kb) Deletion
- Die Fusion des IncHI2 mit dem *bla*<sub>NDM-1</sub>-tragenden IncA/C<sub>2</sub> Plasmid (~450 Megaplasmid)



Das *bla*<sub>NDM-1</sub> Gen ging nicht verloren



Format Abstract

Send to

Antimicrob Agents Chemother. 2019 Jul 25;63(8). pii: e00380-19. doi: 10.1128/AAC.00380-19. Print 2019 Aug.

## The *bla*<sub>NDM-1</sub>-Carrying IncA/C<sub>2</sub> Plasmid Underlies Structural Alterations and Cointegrate Formation *In Vivo*.

Hadziabdic S<sup>1</sup>, Fischer J<sup>1</sup>, Borowiak M<sup>1</sup>, Malorny B<sup>1</sup>, Juraschek K<sup>1</sup>, Kaesbohrer A<sup>1</sup>, Guerra B<sup>2</sup>, Deneke C<sup>1</sup>, Gonzalez-Zorn B<sup>3</sup>, Szabo I<sup>4</sup>.

### Author information

- Department for Biological Safety, German Federal Institute for Risk Assessment (BfR), Berlin, Germany.
- European Food Safety Authority, Parma, Italy.
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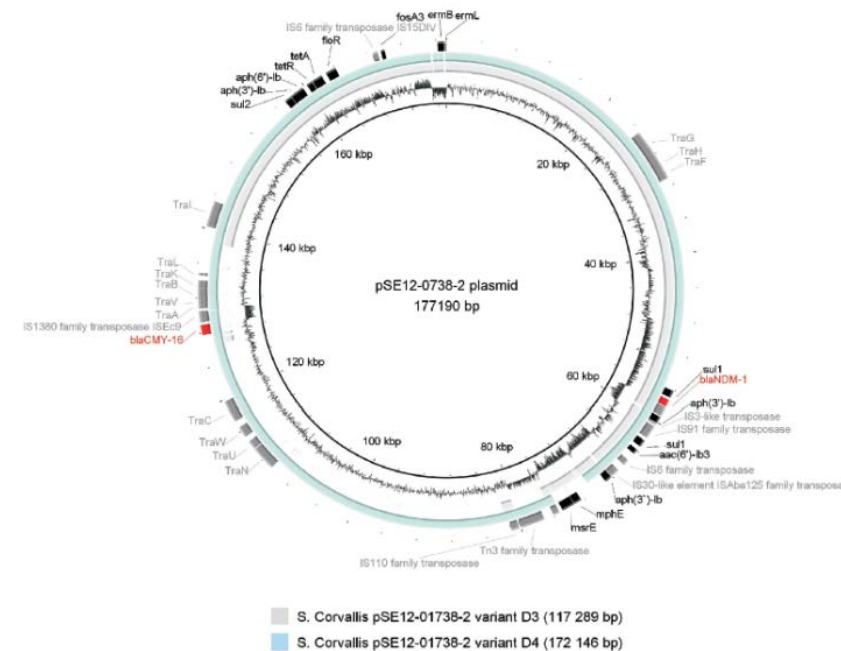


FIG 3 Visualization of the *bla*<sub>NDM-1</sub>-carrying pSE12-01738-2 variants D3 and D4 compared to PacBio RSII reference sequence of pSE12-01738-2 plasmid (GenBank accession number CP027679) using BRIG (6) with resistance genes (red, beta-lactam genes; black, other resistance genes) as well as IS elements, transposase, and *tra* genes (all marked gray).

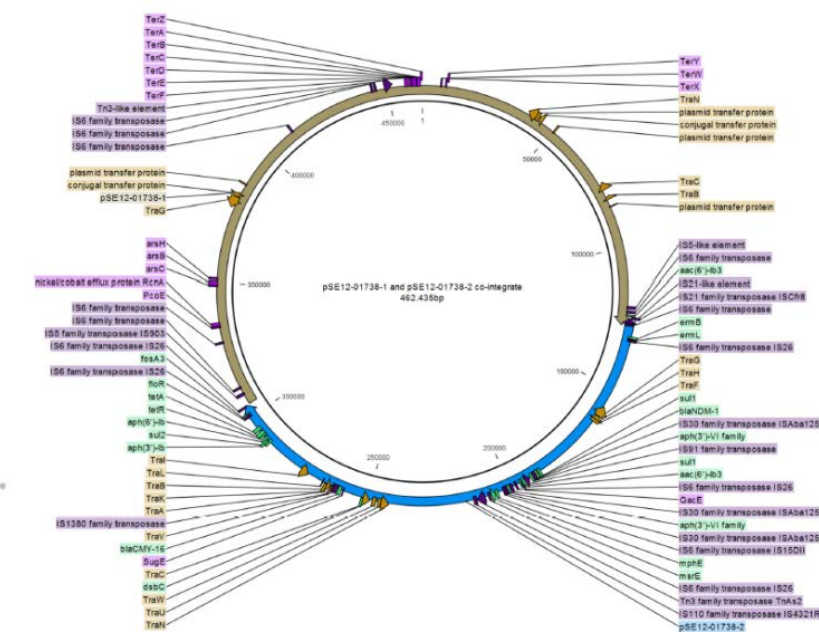


FIG 4 Structure of newly emerged IncHI2 pSE12-01738-1 and IncA/C<sub>2</sub> pSE12-01738-2 co-integrated megaplasmid (462,435 bp). The fusion of IncHI2 pSE12-01738-1 (brown) and IncA/C<sub>2</sub> pSE12-01738-2 plasmid (blue) is shown. Resistance genes are marked green, heavy metal resistance genes are pink, transposase and IS elements are purple, and transfer (*tra*) genes are brown.

# Fazit

- *In vivo* Verbreitung des  $bla_{\text{NDM-1}}$ -tragenden IncA/C<sub>2</sub> Plasmids
  - Unterschiedliche Enterobakterien können das Plasmid aufnehmen
- Das  $bla_{\text{NDM-1}}$ -tragenden IncA/C<sub>2</sub> Plasmid blieb stabil
  - In unterschiedlichen Enterobakterien
  - Im *S. Corvallis* Donor-Stamm
- Das  $bla_{\text{NDM-1}}$  Gen ging trotz Strukturänderung des Plasmids nicht verloren
- All dies geschieht ohne Verwendung von Antibiotika
- Wichtigkeit der Biosicherheitsmaßnahmen
- Einsatz der anderen Antibiotika?

# Danksagung



## Fachgruppe 42

Lebensmittelmikrobiologie, Erreger-Wirt-Interaktionen

- Dr. Istvan Szabo
- Angelina Bloch
- Sophie Irmer
- Johanna Ledwolorz
- Dr. Jennie Fisher
- Martha Brom
- Ernst Junker
- Manuela Rister

## Fachgruppe 43

Epidemiologie, Zoonosen und Antibiotikaresistenz

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- Katharina Juraschek

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- Bea Baumann (Fotos)
- Maria Borowiak

## Fachgruppe 95

Tierhaltung, Aquakultur und Referenzmaterial

- Dr. Stefanie Banneke
- Birgit Frischmuth
- Ursula Barabas

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