Universität Zürich[UZH]

NENT Institute for Food Safety and Hygiene

UCD Institute of Food and Health *in conjunction with* the UCD Centre for Food Safety and the National Zoonoses Committee

Antimicrobial Resistance: A Whole Food Chain Approach

Dublin, 16-Dec-2014

The broader environment and its impact on the dissemination of antimicrobial resistance

Herbert Hächler

Swiss National Centre for Enteropathogenic Bacteria and Listeria (NENT)

Contents

- Resistance dissemination: Theory
- β-lactams, β-lactamases, ESBLs: Basics
- Studies at NENT / ILS Zürich and UCD Dublin: ESBLs along food chain and in the environment
- Besançon: ESBLs from Hospitals / waste water treamtent
- Conclusions

Food production → environment?

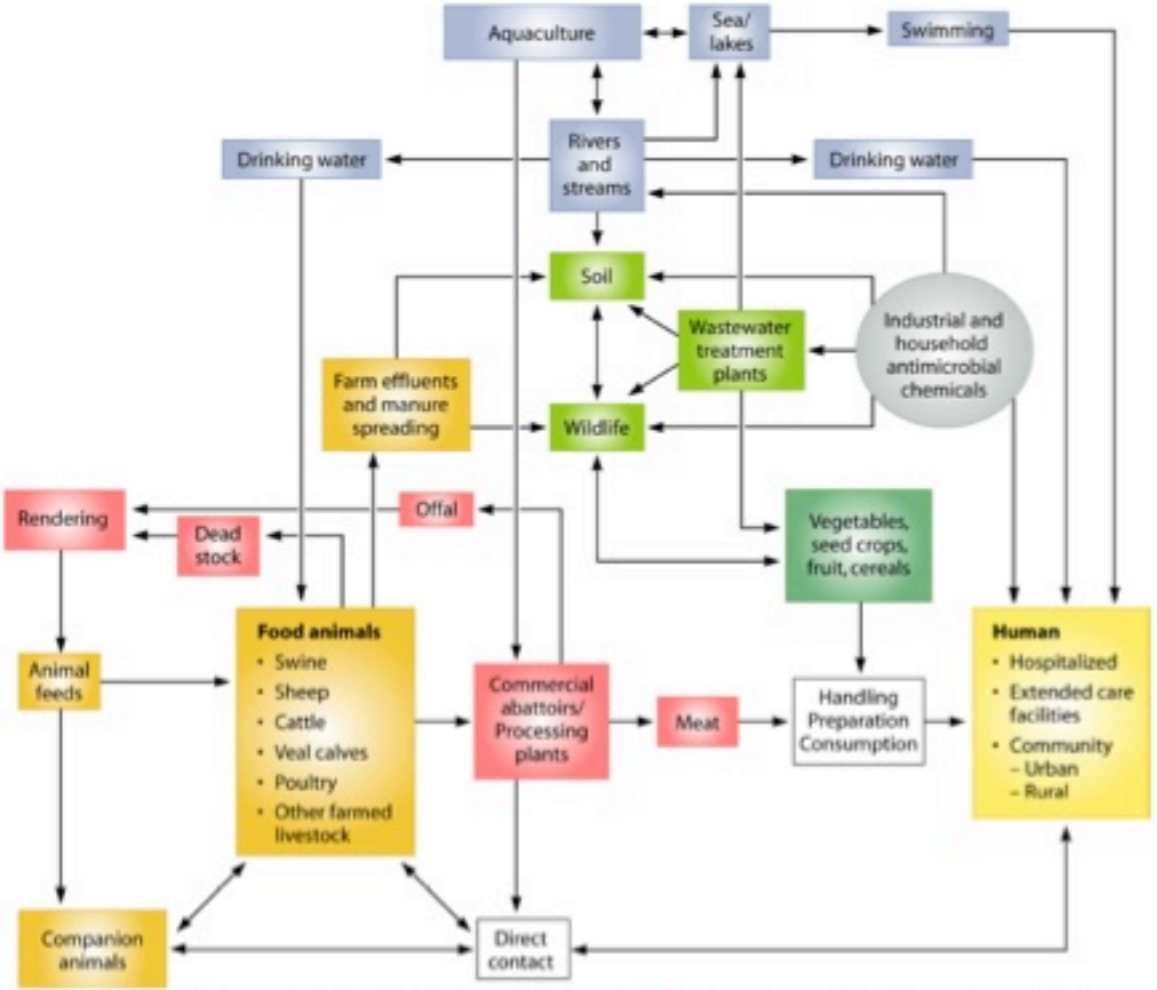

FIG. 4. Dissemination of antibiotics and antibiotic resistance within agriculture, community, hospital, wastewater treatment, and associated environments. (Adapted from reference 49 and reference 83a with permission of the publishers.)

Davies et al. 2010. MMBR **74**:417-433

Contents

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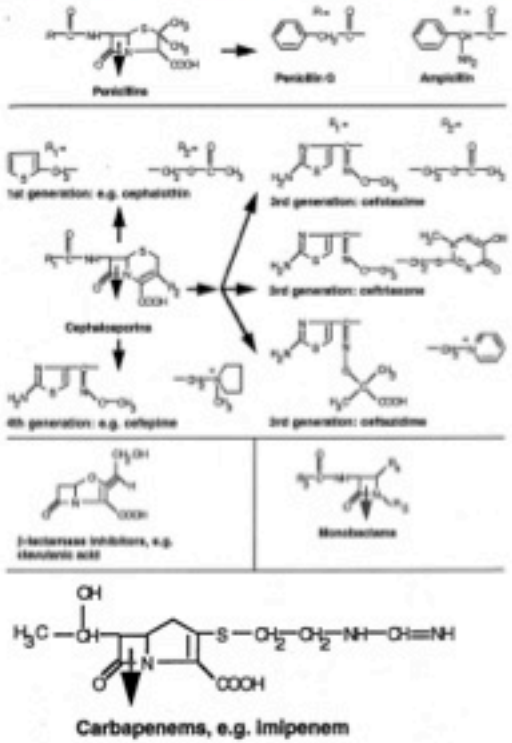
Why beta-lactams?

Extremely important: **β-Lactams**:



- Penicillins
- Inhibitor Combinations
- Cephalosporins
- Carbapenems
- etc.

Why? → Because around 2/3 of human anti-infectious therapies worldwide are still based on β-lactams!

Why ESBLs?

Gram-positive: Methicillin-resistant *Staphylococcus aureus*

→ MRSA

Gram-negative: Producers of extended-spectrum β-lactamase

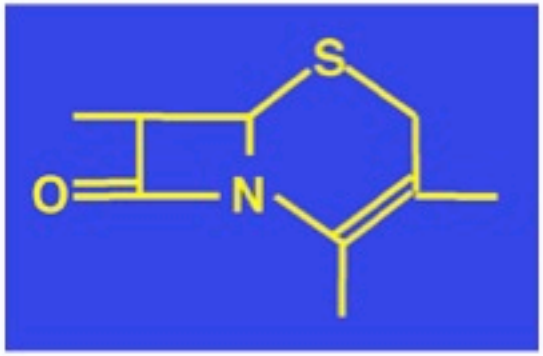
→ ESBL →

Even more abundant!

Corruption of cephalosporins 1: BSBLs

4. Generation:
Cefepime
Cefpirome

1. Generation:
Cephalothin
Cefaloridin



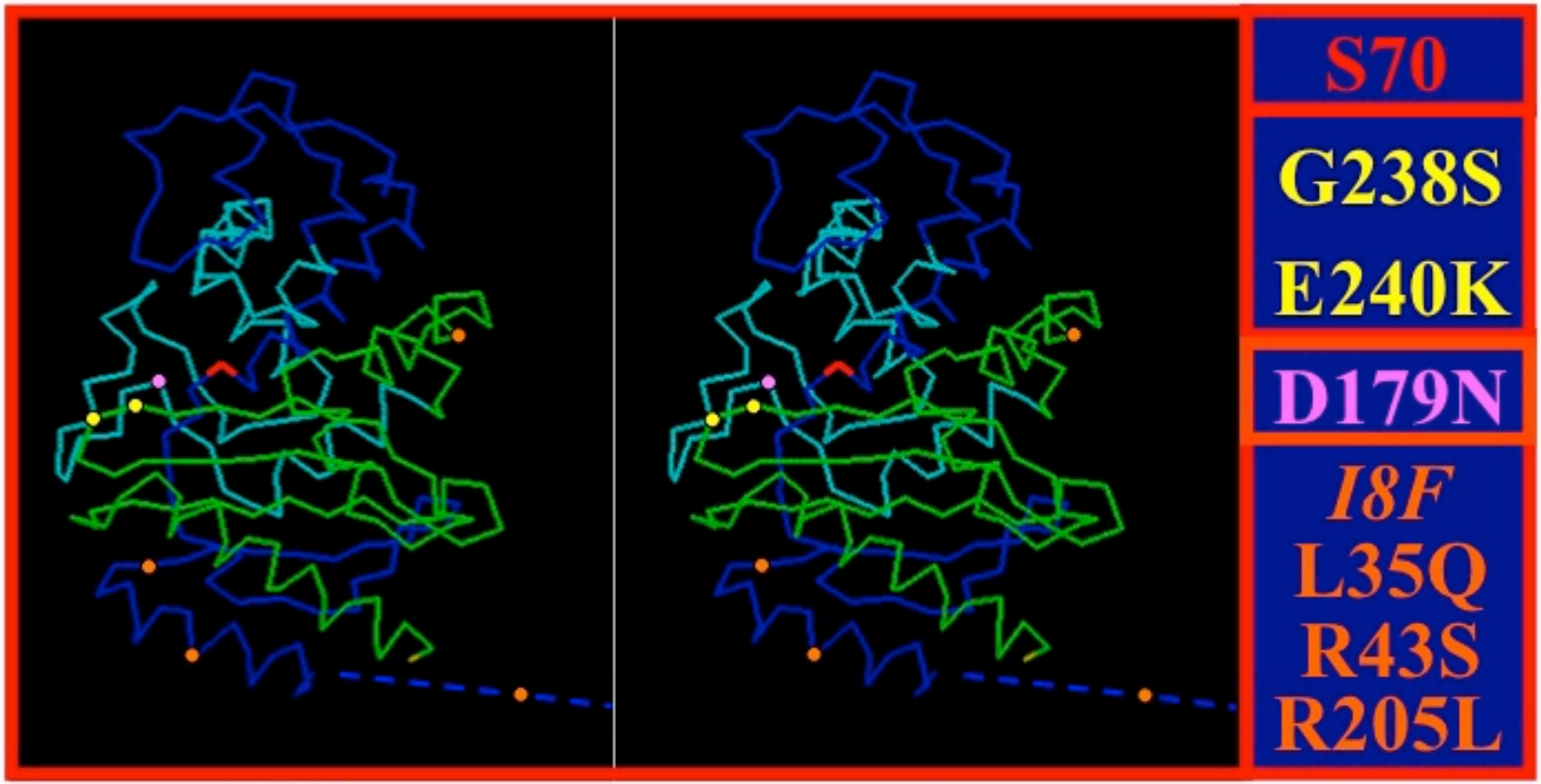
Basic structure

BSBLs

3. Generation:
Cefotaxime
Ceftriaxon
Ceftazidime

2. Generation:
Cefamandole
Cefuroxime

Amino acid substitutions: BSBL →ESBL

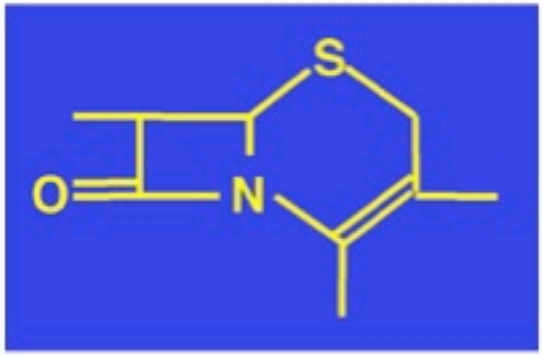


Corruption of cephalosporins 2: ESBLs

4. Generation:
Cefepime
Cefpirome

1. Generation:
Cephalothin
Cefaloridin

ESBLs



Basic structure

BSBLs

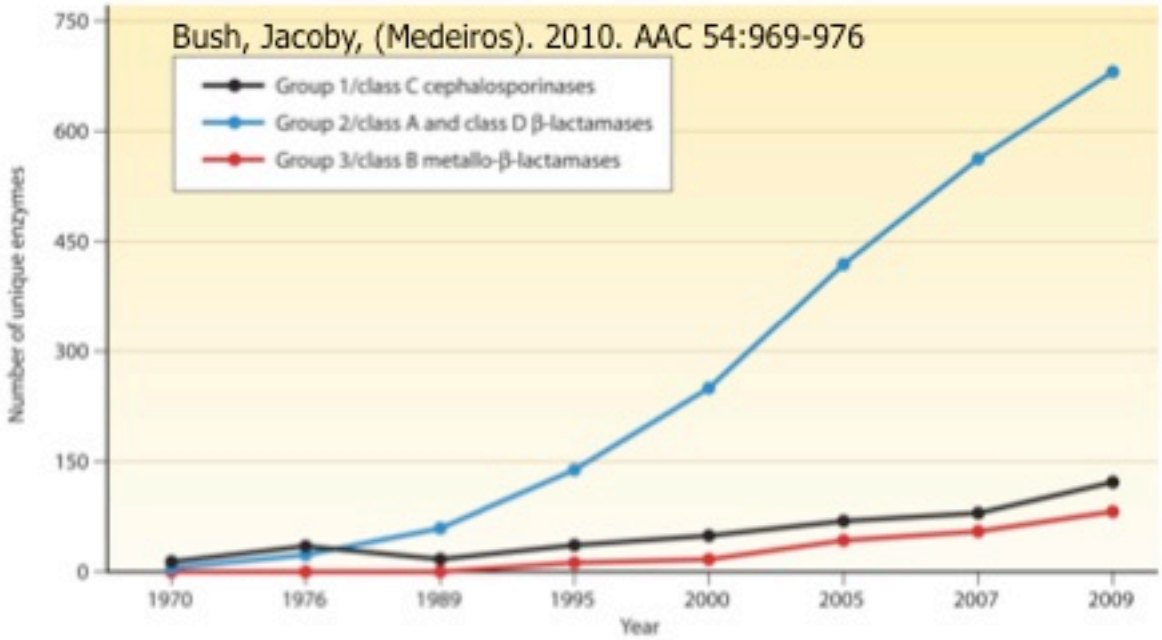
3. Generation:
Cefotaxime
Ceftriaxon
Ceftazidime

2. Generation:
Cefamandole
Cefuroxime

Evolution of ESBL diversity

ESBL: Dissemination

Worldwide!

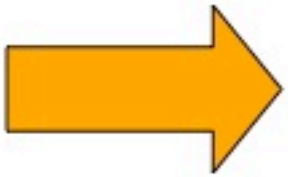


And: ESBLs diversify at a great speed

ESBL: when and where?

1. 1983: SHV-2 (published by German group)
2. 1987: TEM-3 (published by French group)
3. 1993: First time in Switzerland (SHV-11, SHV-12)
4. By 1997: TEM >50, SHV >10: → Worldwide!

5. From ca. 2001: slow displacement of TEM/SHV by CTX-M
6. Until 2005: Mainly nosocomial problem (outbreaks)
7. Since 2006: Steady increase in general community
   [Mesa RJ. 2006. AAC 58:211-215]



World dissemination CTX-M enzymes

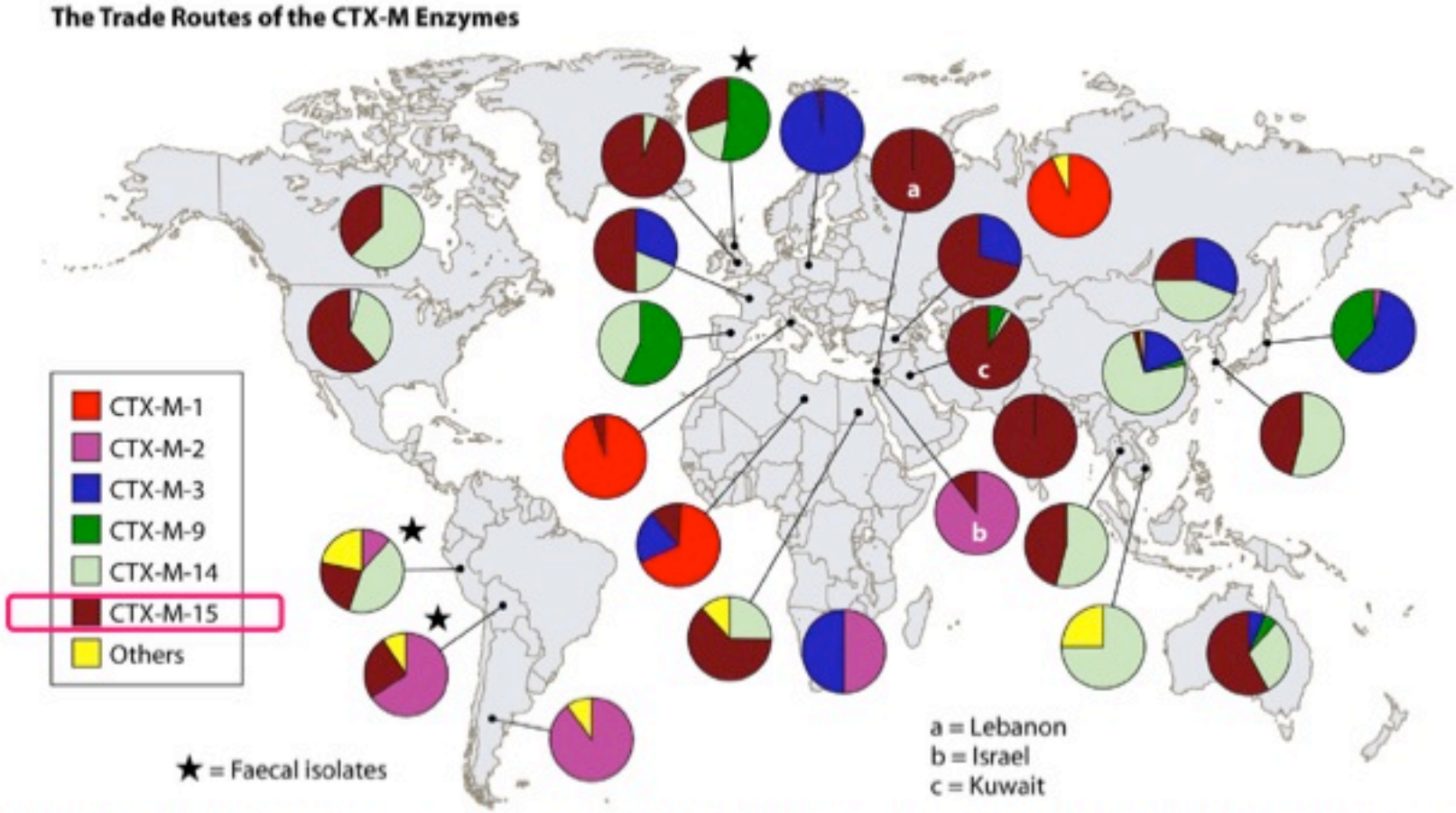


FIG. 3. Worldwide distribution of different classes of CTX-M β-lactamases (first identified in 1989). (Reprinted from reference 71 by permission of Oxford University Press.)

Davies et al. 2010. MMBR **74**:417-433

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ESBL prevalence in food animals

1. Total of ESBL producers	91
2. *Eschericha coli*	89
3. *Citrobacter youngae*	1
4. *Enterobacter cloacae*	1

Origin		n	ESBL producing strains
cattle		124	17 (13.7%)
	calves	63	16 (25.3%)
	others	61	1 (1.6%)
pig		59	9 (15.3%)
chicken		93	59 (63.4%)
sheep		58	5 (8.6%)
	lambs	40	2 (5.0%)
	others	18	3 (16.7%)

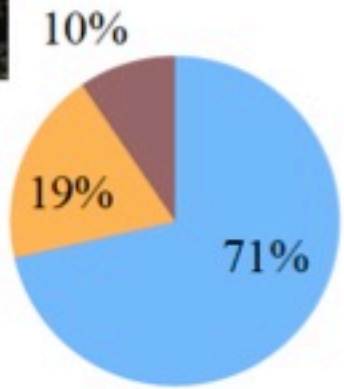
ESBL prevalence in food

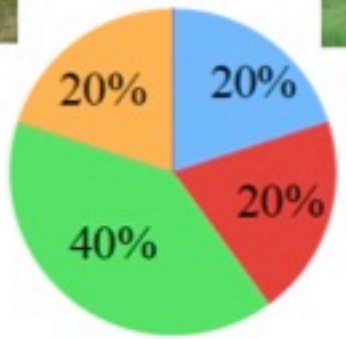
Origin		n	ESBL producing strains
meat (Ground beef and pork)		104	0 (0.0%)
milk		167	1 (0.6%)
	Bulk tank milk	100	0 (0.0%)
	E. coli mastitis milk	67	1 (1.5%)

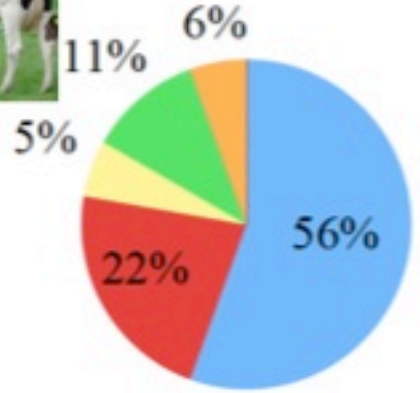
ESBL producers in humans

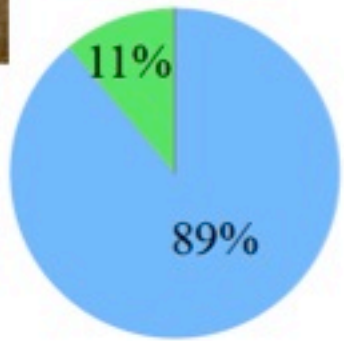
Stool samples from healthy humans screened	586
Positive for ESBL producers	34
%	5.8
Involved bacterial species	1 (*Escherichia coli*)
Expressing additional TEM-1	15 (44 %)

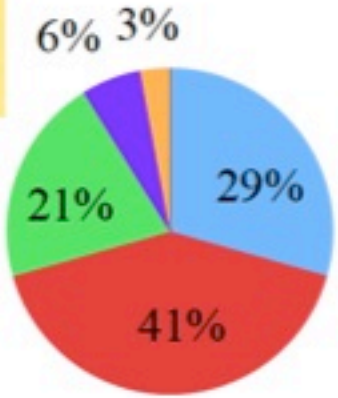
Types of ESBLs

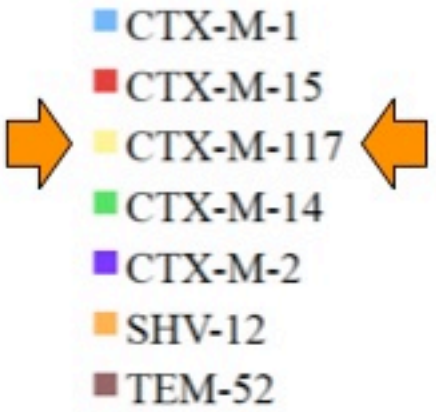












Published at ILS 2011 - 2012

Journal of Food Protection, Vol. 74, No. 3, 2011, Pages 446–449
doi:10.4315/0362-028X.JFP-10-372

Research Note

Fecal Carriage of Extended-Spectrum β-Lactamase–Producing *Enterobacteriaceae* in Swine and Cattle at Slaughter in Switzerland

N. GESER,[1] R. STEPHAN,[1] P. KUHNERT,[2] R. ZBINDEN,[3] U. KAEPPELI,[1] N. CERNELA,[1] AND H. HAECHLER[1]*

AAC Journals.ASM.org

***Antimicrob. Agents Chemother.* 2012, 56(3):1609. DOI: 10.1128/AAC.05539-11.**

Molecular Identification of Extended-Spectrum-β-Lactamase Genes from *Enterobacteriaceae* Isolated from Healthy Human Carriers in Switzerland

Nadine Geser,[a] Roger Stephan,[a] Bozena M. Korczak,[b] Lothar Beutin,[c] and Herbert Hächler[a]*

Occurrence and characteristics of extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae in food producing animals, minced meat and raw milk

BMC Veterinary Research 2012, 8:21 doi:10.1186/1746-6148-8-21

Nadine Geser (nadine.geser@access.uzh.ch)
Roger Stephan (stephanr@fsafety.uzh.ch)
Herbert Hachler (haechlerh@fsafety.uzh.ch)

ESBL in poultry

4

3

2

Total samples from:
Switzerland
Hungary
France

9

Beta-lactamase	Frequency	Frequency (%)
TEM BSBL	2 / 9	22
AmpC type CMY-2	5 / 9	56
ESBL type CTX-M-1	7 / 9	78

ESBL on kitchen cutting boards

1. Cutting boards from the hospital kitchen were sampled after various foods had been cut:
2. The boards had to be washed or even treated in the dish washer to make sure to avoid carry-over
3. No ESBL producers were detected after cutting of either beef, pork, lamb, fish or vegetables
4. ESBL producers were found on 15.6% of the boards after cutting of poultry: 80% of the isolates produced CTX-M-1

Extended-Spectrum β-Lactamase (ESBL)–Producing Enterobacteriaceae: A Threat from the Kitchen

Sarah Tschudin-Sutter, MD, MSc;[1] Reno Frei, MD;[2]
Roger Stephan, DVM;[3] Herbert Hächler, PhD;[3]
Danica Nogarth;[1] Andreas F. Widmer, MD, MSc[1]

INFECTION CONTROL AND HOSPITAL EPIDEMIOLOGY MAY 2014, VOL. 35, NO. 5

Journal of Food Protection, Vol. 77, No. 1, 2014, Pages 112–115
doi:10.4315/0362-028X.JFP-13-120

Research Note

Characteristics of Extended-Spectrum Cephalosporin-Resistant *Escherichia coli* Isolated from Swiss and Imported Poultry Meat

H. ABGOTTSPON,[1] R. STEPHAN,[1]* C. BAGUTTI,[2] P. BRODMANN,[2] H. HÄCHLER,[1] AND K. ZURFLUH[1]

Vertical transmission of ESBLs in chickens

frontiers in MICROBIOLOGY

ORIGINAL RESEARCH ARTICLE
published: 30 September 2014
doi: 10.3389/fmicb.2014.00519

Vertical transmission of highly similar *bla*$_{CTX-M-1}$-harboring IncI1 plasmids in *Escherichia coli* with different MLST types in the poultry production pyramid

Katrin Zurfluh[1], Juan Wang[2], Jochen Klumpp[3], Magdalena Nüesch-Inderbinen[1], Séamus Fanning[2] and Roger Stephan[1]*

[1] Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zürich, Switzerland
[2] UCD Centre for Food Safety, School of Public Health, Physiotherapy and Population Science, UCD Centre for Molecular Innovation and Drug Discovery, University College Dublin, Dublin, Ireland
[3] Institute of Food, Nutrition and Health, Swiss Federal Institute of Technology in Zürich, Zürich, Switzerland

Edited by:
Kornelia Smalla, Julius Kühn-Institut – Federal Research Centre for Cultivated Plants, Germany

Reviewed by:
Yuji Morita, Aichi Gakuin University, Japan
Siddharth Kaushal Tripathi, University of Mississippi, USA

***Correspondence:**
Roger Stephan, Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Winterthurerstr 272, CH-8057 Zurich, Switzerland
e-mail: stephanr@fsafety.uzh.ch

Objectives: The purpose of this study was to characterize sets of extended-spectrum β-lactamases (ESBL)-producing *Enterobacteriaceae* collected longitudinally from different flocks of broiler breeders, meconium of 1-day-old broilers from theses breeder flocks, as well as from these broiler flocks before slaughter.

Methods: Five sets of ESBL-producing *Escherichia coli* were studied by multi-locus sequence typing (MLST), phylogenetic grouping, PCR-based replicon typing and resistance profiling. The *bla*$_{CTX-M-1}$-harboring plasmids of one set (pHV295.1, pHV114.1, and pHV292.1) were fully sequenced and subjected to comparative analysis.

Results: Eleven different MLST sequence types (ST) were identified with ST1056 the predominant one, isolated in all five sets either on the broiler breeder or meconium level. Plasmid sequencing revealed that *bla*$_{CTX-M-1}$ was carried by highly similar IncI1/ST3 plasmids that were 105 076 bp, 110 997 bp, and 117 269 bp in size, respectively.

Conclusions: The fact that genetically similar IncI1/ST3 plasmids were found in ESBL-producing *E. coli* of different MLST types isolated at the different levels in the broiler production pyramid provides strong evidence for a vertical transmission of these plasmids from a common source (nucleus poultry flocks).

Keywords: *E. coli*, plasmid sequencing, CTX-M-1, poultry production pyramid, IncI1, conjugation

1. Most *E. coli* with variable genetic background
2. IncI1 plasmids highly similar
3. Evidence for vertical transmisson of IncI1::*bla*$_{CTX-M-1}$ plasmids from nucleus poultry flocks

Vertical transmission of ESBLs in chickens

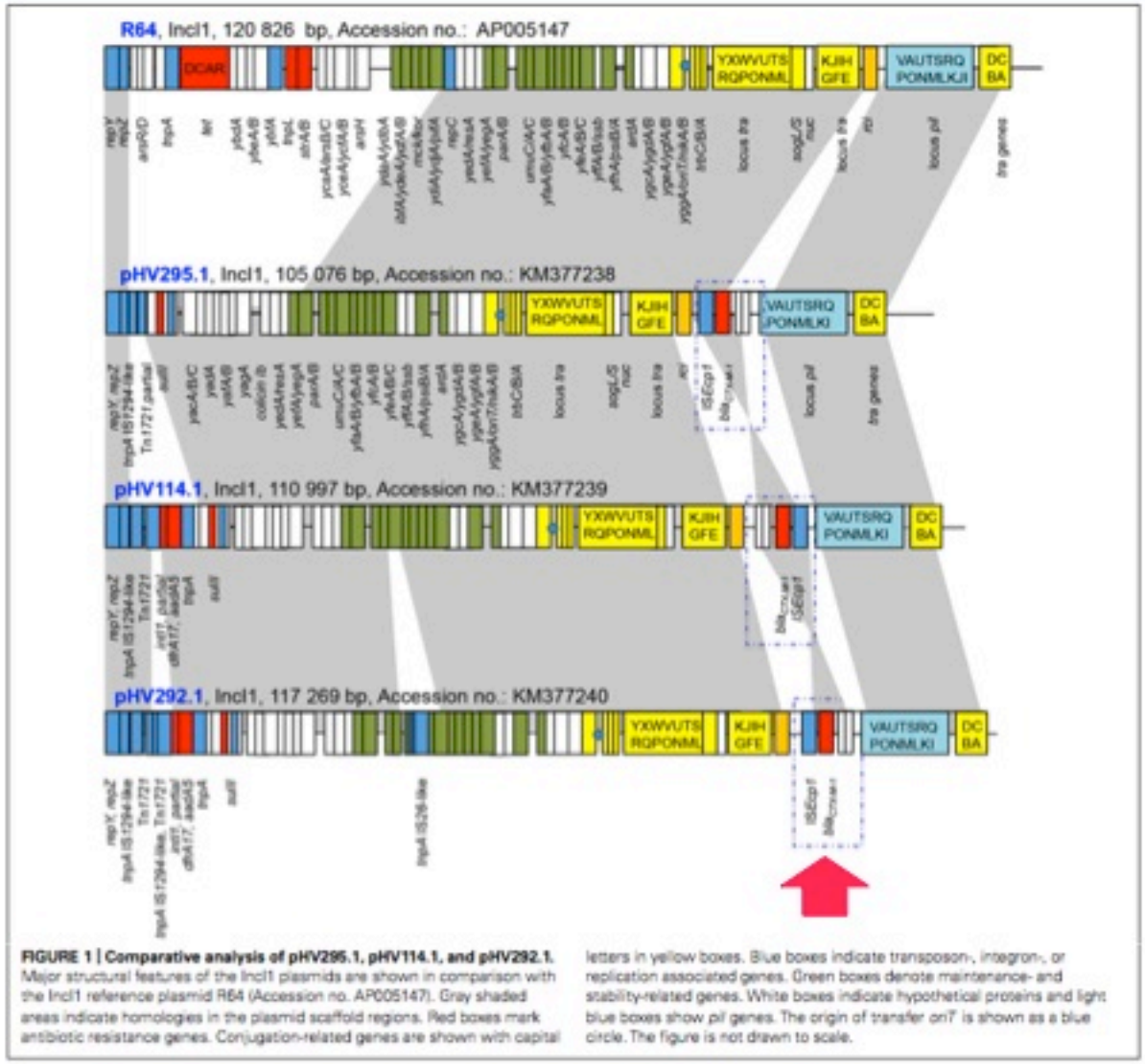
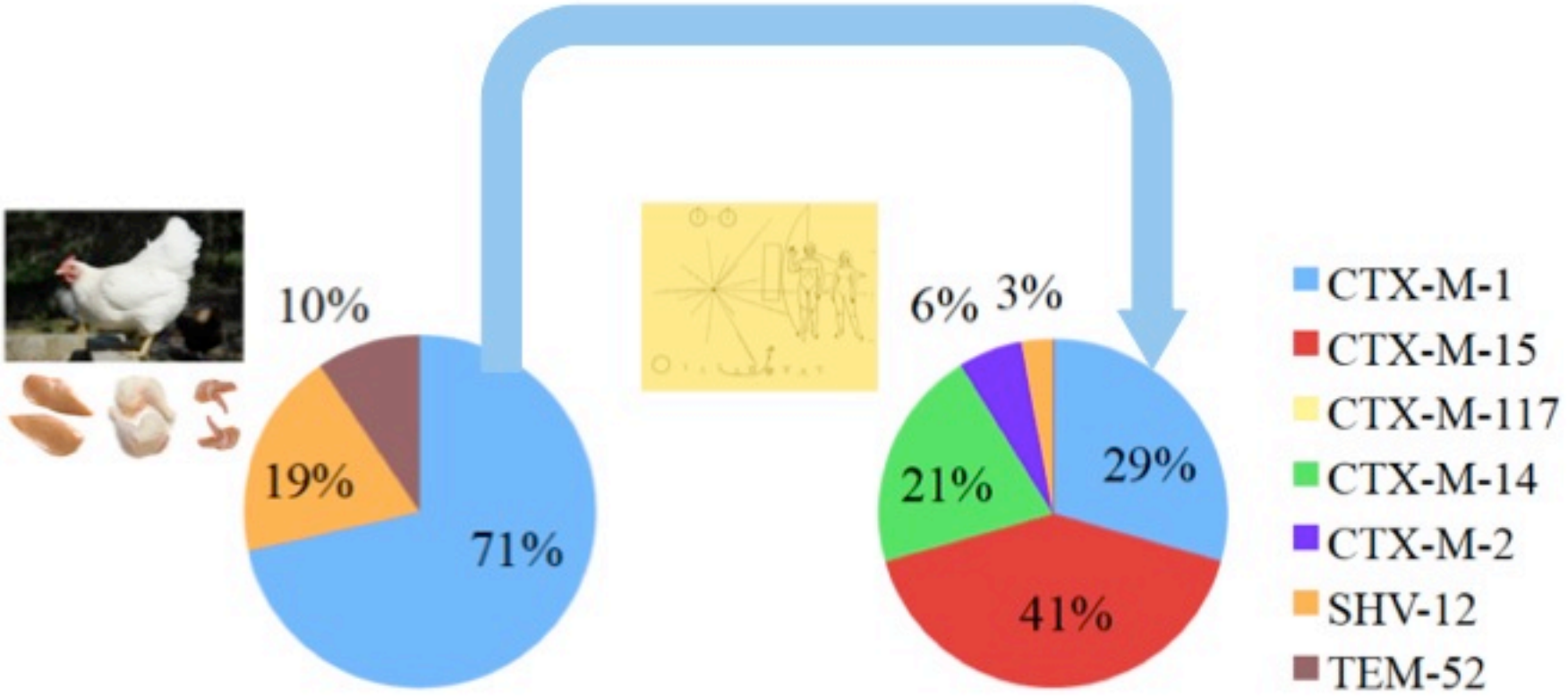


FIGURE 1 | Comparative analysis of pHV295.1, pHV114.1, and pHV292.1. Major structural features of the IncI1 plasmids are shown in comparison with the IncI1 reference plasmid R64 (Accession no. AP005147). Gray shaded areas indicate homologies in the plasmid scaffold regions. Red boxes mark antibiotic resistance genes. Conjugation-related genes are shown with capital letters in yellow boxes. Blue boxes indicate transposon-, integron-, or replication associated genes. Green boxes denote maintenance- and stability-related genes. White boxes indicate hypothetical proteins and light blue boxes show *pil* genes. The origin of transfer oriT is shown as a blue circle. The figure is not drawn to scale.

ESBL in poultry



Plausible explanation for human burden due to CTX-M-1 expressing *E. coli*

ESBLs in feral birds

Feral rock pigeon

Columba livia

Great cormorant

Phalacrocorax carbo

ESBLs in feral birds: Results

Sample size:

Pigeons 298 Cormorants 30

Strain	Origin	β-Lactamase	MLST	Phylogroup
W117E	Pigeon	CTX-M-15	N/D	B2
W117C	Pigeon	CMY-2	ST457	D
W132	Pigeon	CMY-2	ST457	D
W265	Pigeon	CMY-2	ST457	D
W34	Cormorant	CTX-M-15	ST120	B1
W43	Cormorant	CTX-M-27	ST131	B2

ESBLs in feral birds: Reference

Katrin Zurfluh
Magdalena Nüesch-Inderbinen
Roger Stephan
Herbert Hächler*
Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 272, CH-8057 Zurich, Switzerland

Higher-generation cephalosporin-resistant *Escherichia coli* in feral birds in Switzerland

Letters to the Editor / International Journal of Antimicrobial Agents 41 (2013) 292–299

doi:10.1016/j.ijantimicag.2012.11.005

ESBL in wild animal!!

Total fecal samples analysed:

235

Positive: 1 Roe deer hunted in Rotkreuz ZG

Sequenced ESBL: → CTX-M-1

Doctoral thesis: Tobias Obwegeser

Roe deer: Reference

Schweizer Archiv für Tierheilkunde
R. Stephan, H. Hächler, Band 154, Heft 11, November 2012, 475–478
DOI 10.1024/0036-7281/a000390

ESBL producing *E. coli* in wild ruminants

Discovery of extended-spectrum β-lactamase producing *Escherichia coli* among hunted deer, chamois and ibex

R. Stephan, H. Hächler

Institute for Food Safety and Hygiene, University of Zurich

ESBL in fresh water fish

64 Whitefish *Coregonus lavaretus*

33 Perch *Perca fluvuiatilis*

29 Roach *Rutilus rutilus*

6 Brown Trout *Salmo trutta*

139 Samples from:

Lake of Zurich

Lake of Thun

4 Pike *Esox lucius*

1 Bream *Abramis brama*

1 Tench *Tinca tinca*

1 Sunfish *Centrarchidae*

ESBL in fresh water fish

- 26 / 139 fish (18.7%) yielded 33 carriers of pAmpC or ESBL: 23 (16.5%) from lake Zurich, 3 (2.2%) from lake Thun

- Among the 33 strains, the following *bla* genes were found:

13 *bla*CTX-M-15	7 *bla*CTX-M-27	4 *bla*CTX-M-1
4 *bla*CTX-M-14	2 *bla*CTX-M-24	2 *bla*SHV-12
1 *bla*CMY-2		

ESBL in fresh water fish

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LETTER TO THE EDITOR

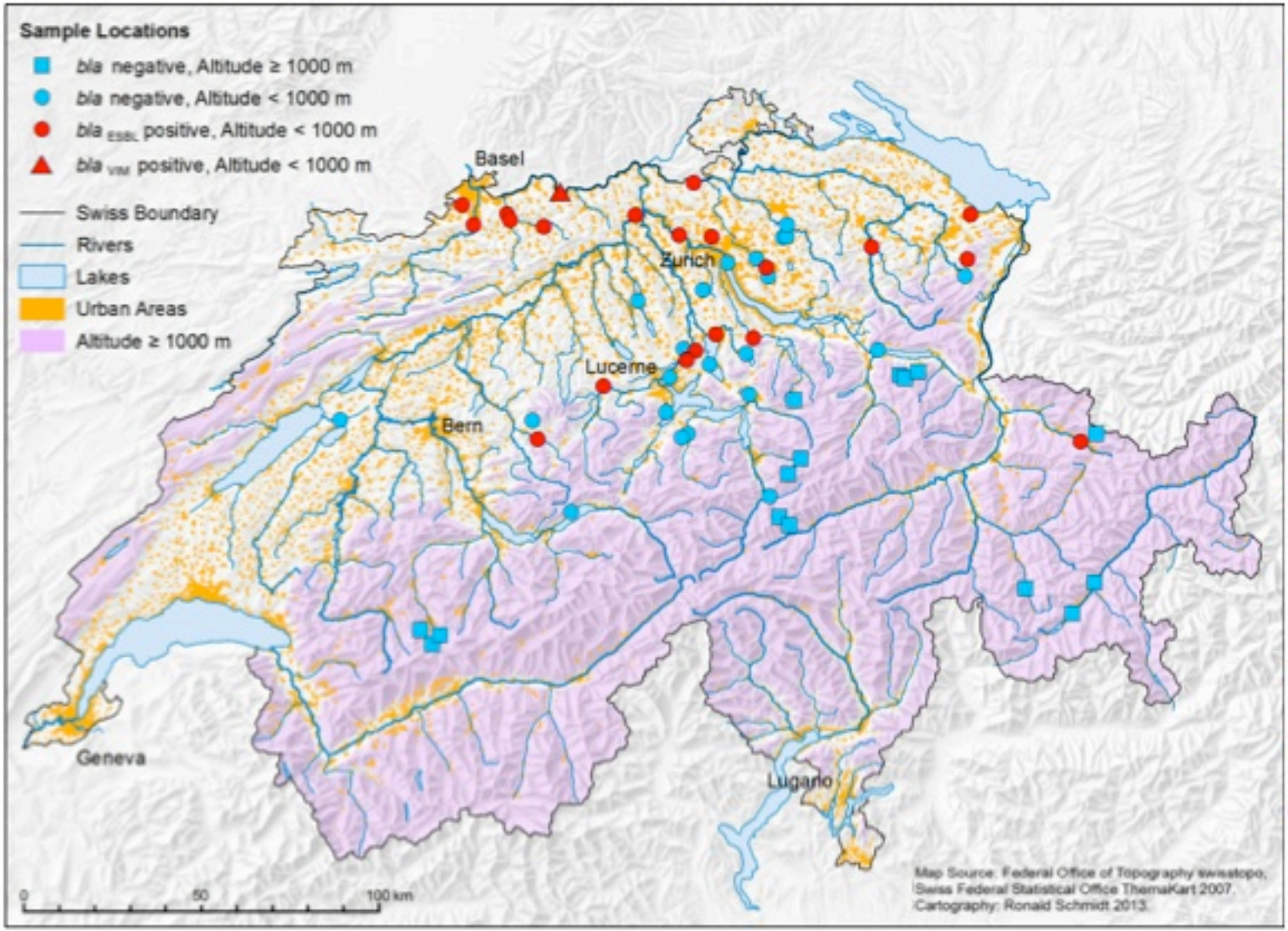
Enterobacteriaceae with Extended-Spectrum- and pAmpC-Type β-Lactamase-Encoding Genes Isolated from Freshwater Fish from Two Lakes in Switzerland

Helga Abgottspon, Magdalena T. Nüesch-Inderbinen, Katrin Zurfluh, Denise Althaus, Herbert Hächler, Roger Stephan

Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, Zurich, Switzerland

2482 aac.asm.org Antimicrobial Agents and Chemotherapy p. 2482–2484 April 2014 Volume 58 Number 4

3rdGen-Ceph^R and Carb^R in surface waters



Surface waters: 58

Rivers: 40

Lakes: 18

Positive for ESBL or carbapenemase: 21/58 (36.2%)

Total isolates: 75

ESBL-producer: 74

Carba-producer: 1

Analogy WWII

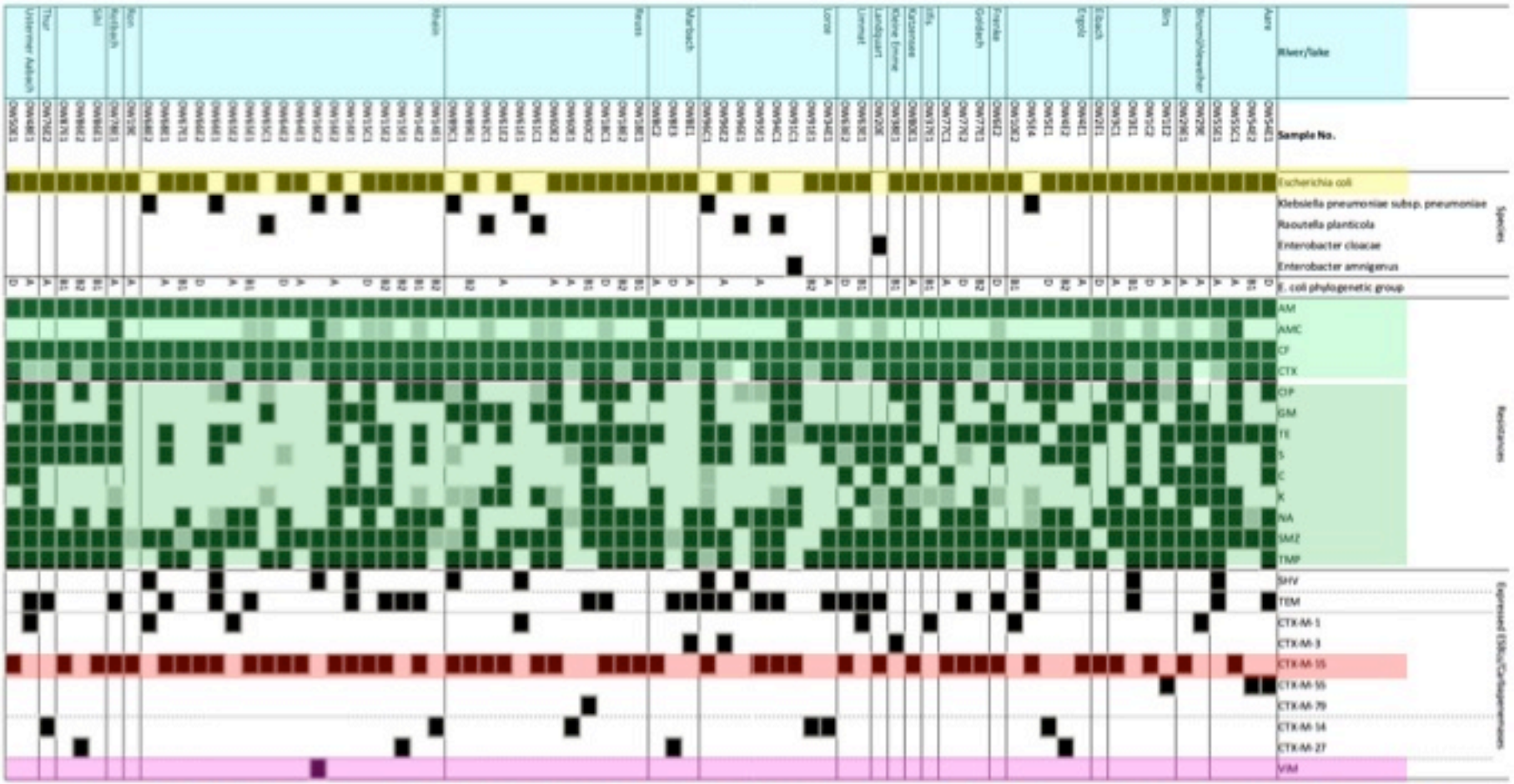
Sir Winston Churchill: House of Commons June-18, 1940

„What General Weygand called The Battle of France is over. I expect that The Battle of Britain is about to begin"

http://www.winstonchurchill.org/learn/speeches/speeches-of-winston-churchill/122-their-finest-hour

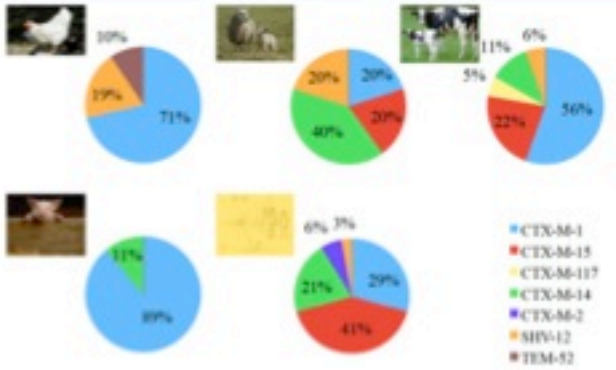
Analogy: The battle against ESBLs is over. I expect that the battle against carbapenemases is about to begin

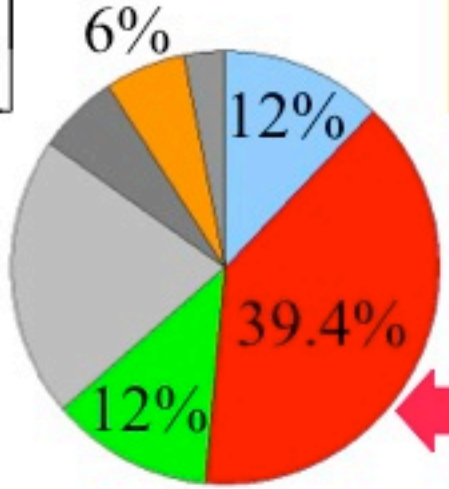
3rdGen-CephR and CarbR in surface waters

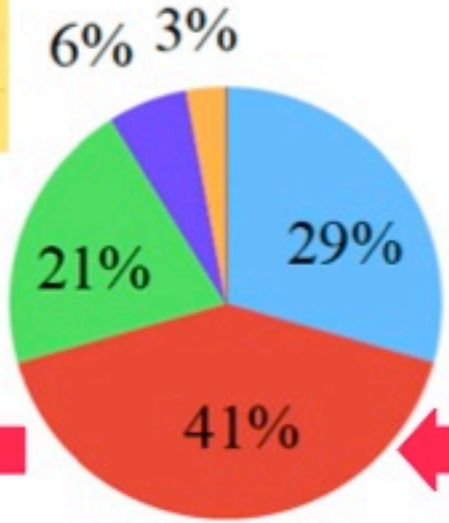


Total isolates: 75 ESBL-producer: 74 (CTX-M-15: **46 (62%)** / Carba-producer: 1

Origin of CTX-M-15 producers in water??







CTX-M-1
CTX-M-15
CTX-M-117
CTX-M-14
CTX-M-2
SHV-12
TEM-52

(CTX-M-15: **46 (62%)**

Surface waters: Reference

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Characteristics of Extended-Spectrum β-Lactamase- and Carbapenemase-Producing *Enterobacteriaceae* Isolates from Rivers and Lakes in Switzerland

Katrin Zurfluh, Herbert Hächler, Magdalena Nüesch-Inderbinen, Roger Stephan
Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

May 2013 Volume 79 Number 9 Applied and Environmental Microbiology p. 3021–3026 aem.asm.org 3021

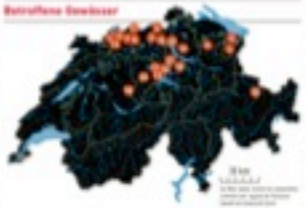
General-Media Coverage

5 SchweizNachrichten

Killer-Keime in Gewässern

Resistente Fäkalbakterien Ursache von nur schwer behandelbaren Infektionen

Betroffene Gewässer



„Art work“ for detailed plasmid comparison

Frontiers in MICROBIOLOGY

ORIGINAL RESEARCH ARTICLE

Molecular characterization of bla_{ESBL}-harboring conjugative plasmids identified in multi-drug resistant *Escherichia coli* isolated from food-producing animals and healthy humans

Juan Wang[1], Roger Stephan[2], Maria Kaczmarczyk[1], Qingqiang Yan[1], Herbert Hächler[2] and Séamus Fanning[1,3]

Journal of Antimicrobial Chemotherapy Advance Access published June 11, 2014

Journal of Antimicrobial Chemotherapy

J Antimicrob Chemother doi:10.1093/jac/dku196

Nucleotide sequences of 16 transmissible plasmids identified in nine multidrug-resistant *Escherichia coli* isolates expressing an ESBL phenotype isolated from food-producing animals and healthy humans

Juan Wang[1], Roger Stephan[2], Karen Power[1], Qingqiang Yan[1], Herbert Hächler[2] and Séamus Fanning[1,3]*

MICROBIAL DRUG RESISTANCE Volume 00, Number 00, 2014 © Mary Ann Liebert, Inc. DOI: 10.1089/mdr.2014.0055

EPIDEMIOLOGY

A Novel Tn*3*-Like Composite Transposon Harboring bla_{VIM-1} in *Klebsiella pneumoniae* spp. *pneumoniae* Isolated from River Water

Katrin Zurfluh[1], Karen A. Power[1,2], Jochen Klumpp[3], Juan Wang[1,2], Séamus Fanning[1,2] and Roger Stephan[1]

ORIGINAL RESEARCH ARTICLE

Front. Microbiol., 30 October 2014 | doi: 10.3389/fmicb.2014.00555

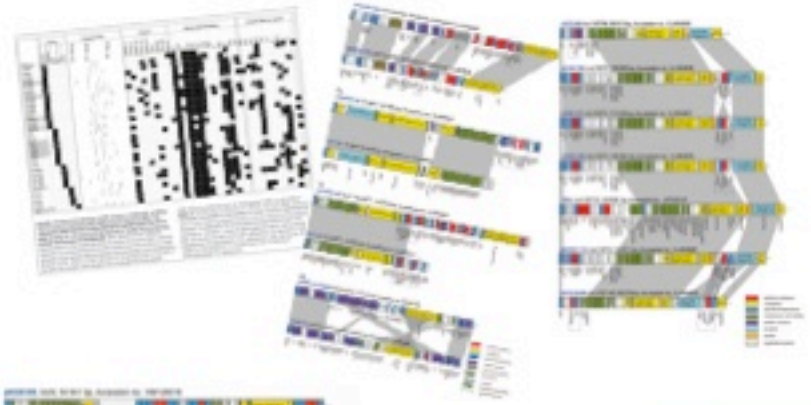
Replicon typing of plasmids carrying $bla_{CTX-M-1}$ in *Enterobacteriaceae* of animal, environmental and human origin

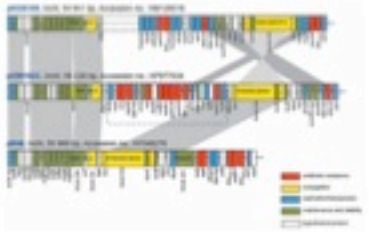
Katrin Zurfluh, Gianna Jakobi, Roger Stephan*, Herbert Hächler and Magdalena Nüesch-Inderbinen

Juan Wang[1], Roger Stephan[2], Katrin Zurfluh[2], Herbert Hächler[2] and Séamus Fanning[1,3]*

Characterization of the genetic environment of bla_{ESBL} genes, integrons and toxin-antitoxin systems identified on large transferrable plasmids in multi-drug resistant *Escherichia coli*

Frontiers in Microbiology: *In press*





UCD DUBLIN

Universität Zürich[UZH]

„Art work“ for detailed plasmid comparison

Poultry-derived plasmids with *bla*$_{CTX-M-1}$ → highly linked to IncI1/ ST3, human-, cattle and pig-derived ones to a lesser extent plus to IncI1/ST1

Human-derived plasmids with *bla*$_{CTX-M-15}$ → predominantly linked to IncF, and, to a lesser extent, to IncI1, IncK and IncR

Moreover, many conjugative plasmids carrying *bla*$_{CTX-M}$ genes express Toxin/Antitoxin systems for stability

Contents

- Resistance dissemination: Theory
- β-lactams, β-lactamases, ESBLs: Basics
- Studies at NENT / ILS Zürich and UCD Dublin: ESBLs along food chain and in the environment
- Besançon: ESBLs from Hospitals / waste water treamtent
- Conclusions

Hospital sewage, France

http://www.limmattalerzeitung.ch/limmattal/zuerich/dank-klaerschlamm-verwertungsanlage-phosphor-gewinnen-12587797

MAJOR ARTICLE

Wastewater Treatment Plants Release Large Amounts of Extended-Spectrum β-Lactamase–Producing *Escherichia coli* Into the Environment

Caroline Bréchet,[1] Julie Plantin,[1] Marlène Sauget,[1] Michelle Thouverez,[1] Daniel Talon,[1] Pascal Cholley,[1] Christophe Guyeux,[2] Didier Hocquet,[1] and Xavier Bertrand[1]

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(See the Editorial Commentary by Griffiths and Barza on pages 1666–7.)

Results and conclusions in Besançon

1. Total *E. coli* in waste water (/ml): Urban > hospital
   $7.5x10^5 > 3.5x10^5$ x 2.2
2. ESBL *E. coli* in waste water (/ml): Urban < hospital
   $0.8x10^3 < 27x10^3$ x 34
3. Elimination total *E. coli* in WWTPlant: 98%
4. Elimination ESBL *E. coli* in WWTPlant: 94%

→ **Relative enrichment of ESBL *E. coli* by WWTPlant !!**

5. Daily release of ESBL *E. coli* into river Doubs $>600x10^8$
5. ESBL *E. coli* in sludge (fertilizer) from WWTP $2.6x10^5$/g

http://www.medscape.com/viewarticle/824743

Editorial / Medscape digest

When the investigators tested isolates for antibiotic susceptibility, they found that the ESBLEC in the hospital wastewater were more resistant to antibiotics than those in the urban wastewater, particularly to ceftazidime ($P < .001$) and ofloxacin ($P < .001$).

"Our results suggest that there is a need for improvements in the monitoring of antibiotic-resistant microorganisms of human origin in effluent," they conclude.

In an editorial commentary accompanying the study, Jeffrey K. Griffiths, MD, MPH, from the Department of Public Health and Community Medicine, Tufts University, Boston, Massachusetts, and Michael Barza, MD, from the Steward Carney Hospital, Tufts University School of Medicine, emphasize that effective treatment of hospital wastewater should be a key component in efforts to stem antibiotic resistance.

http://www.medscape.com/viewarticle/824743

France → Switzerland

ESBLs in healthy humans: **France** 6%

Bréchet et al. 2014. CID **58**:1658-1665; Nicolas-Chanoine et al. 2013. JAC **68**:562-568

Universität Zürich[UZH]

NENT Institute for Food Safety and Hygiene

ESBL producers in humans

Switzerland

Stool samples from healthy humans screened	586
Positive for ESBL producers	34
%	5.8
Involved bacterial species	1 (*Escherichia coli*)
Expressing additional TEM-1	15 (44 %)

21.05.2014 ESBL entlang der Lebensmittelkette. H. Hächler Seite 35

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Conclusions

ESBL producing *Enterobacteriaceae* in Switzerland are to be found in patients, healthy humans, food, farm animals, wild fish, birds and mammals as well as in surface waters

There is strong evidence for transmission of CTX-M-1 producers between chicken (products) and humans

CTX-M-15 is the most frequent ESBL in humans, in WWTPs, and in surface waters

Although located on conjugative plasmids of various Inc groups, $bla_{CTX\text{-}M}$ genes were most often associated with transposable elements such as ISEcp1 or IS26 suggesting common ancestry

The reservoir of CTX-M-15 producers is as yet unknown, but preliminary results seem to suggest vegetable foods

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