Extended Spectrum Beta-Lactamases (ESBLs)

Cindy Dierikx

CRL-DTU molecular course 7 november 2011

CENTRAL VETERINARY INSTITUTE
WAGENINGEN UR

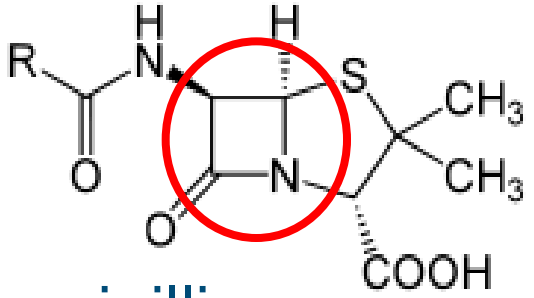
Introduction

- Studied veterinary medicine in Utrecht (NL)
- Worked at small animal practice for one year
- Started at Central Veterinary Institute in 2007 as veterinary microbiologist in training
- PhD on ESBLs in broilers in NL

Outline

- Background
  - What are beta-lactam antibiotics?
  - Resistance to beta-lactam antibiotics
- ESBLs and AmpC
  - classification
  - Why are they important?
- Detection of ESBL/AmpC producing isolates
  - Phenotypic tests
  - Molecular tests
- (Results of surveillance on ESBLs in broilers in the Netherlands)

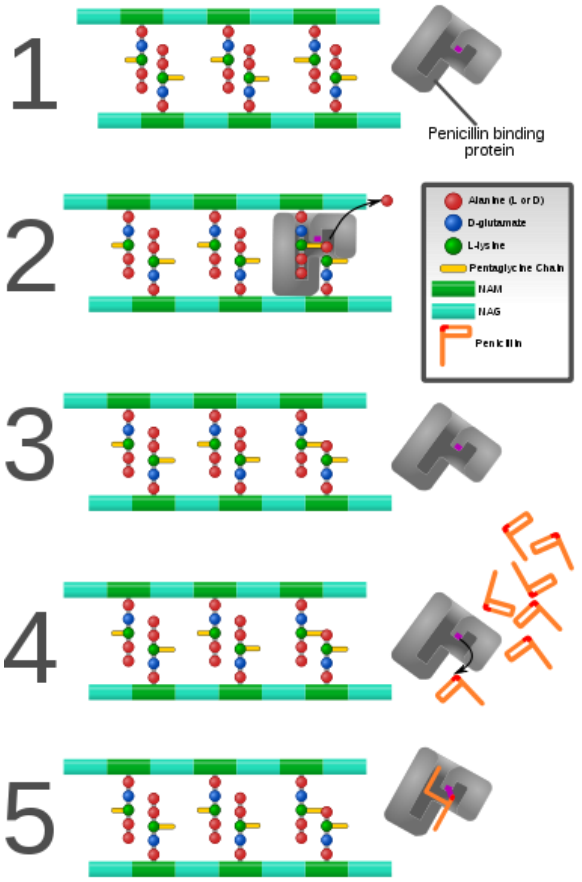
Beta-lactam antibiotics



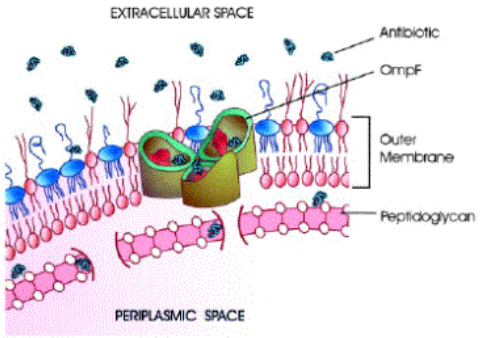
- Penams, or penicillins (e.g. benzylpenicillin, ampicillin)
- Cephems, cephalosporin derivatives (all generations cephalosporins)
- Cephamycins (e.g. cefoxitin)
- Monobactams (e.g. aztreonam)
- Carbapenems and penems (e.g. imipenem, faropenem)

Beta-lactam antibiotics

- Interfere with biosynthesis of peptidoglycan in the bacterial cell wall
- Resistance to beta-lactam antibiotics by:
  - Mutations in outer membrane porins
  - Efflux pumps
  - Mutations in Penicillin Binding Protein
  - Production of beta-lactam hydrolysing enzymes (beta-lactamases)



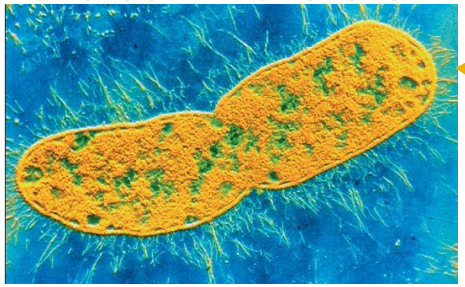
Source: wikipedia

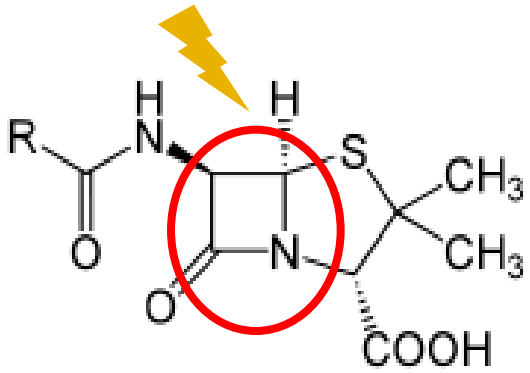


Scheme of the antibiotic pathway. In order to reach their target the antibiotic molecules must permeate the outer cell membrane of the bacteria(7).

Gram negative bacteria

What are beta-lactamases?





- Enzymes produced by gram-negative and gram-positive bacteria
- Hydrolyze beta-lactam ring
- Natural occurring as chromosomally mediated beta-lactamases
- Protected the bacteria from beta-lactam producing soil organisms in the environment (hypothesis)
- In Enterobaceriaceae beta-lactamases occurred that hydrolyse Extended Spectrum Cephalosporins (3th and/or 4th generation cephalosporins)

Beta-lactamase classification scheme

Table 1
Modified classification scheme of β-lactamases (according to Ambler, 1980).

	β-lactamase-class	β-lactamases	Important examples	Preferential occurrence	Important phenotypical resistance traits[a]
Serine-β-lactamases	A	Broad-spectrum β-lactamases	TEM-1, TEM-2 SHV-1, SHV-11	Enterobacteriaceae and nonfermenters[b]	ampicillin, cephalotin
		ESBL TEM-type	TEM-3, TEM-52		penicillins, 3rd gen. cephalosporins
		ESBL SHV-type	SHV-5, SHV-12		
		ESBL CTX-M-type	CTX-M-1, CTX-M-15		
		Carbapenemases	KPC, GES, SME		all β-Lactams[c]
	C	AmpC cephamycinases (chromosomal-encoded)	AmpC	Enterobacter spp. Citrobacter spp.	cephamycins (cefoxitin), 3rd gen. cephalosporins
	D	AmpC cephamycinases (plasmid-encoded)	CMY, DHA, MOX FOX, ACC,	Enterobacteriaceae	cephamycins (cefoxitin), 3rd gen. cephalosporins
		Broad-spectrum β-lactamases	OXA-1, OXA-9	Enterobacteriaceae; A. baumannii	oxacillin, ampicillin cephalotin
		ESBL OXA-type	OXA-2, OXA-10		penicillins, 3rd gen. cephalosporins
		Carbapenemases; Carbapenemases	OXA-48; OXA-23,-24,-58		ampicillin, imipenem; all β-lactams[c]
Metallo-β-lactamases	B	Metallo-β-lactamases (Carbapenemases)	VIM IMP	Enterobacteriaceae and nonfermenters	all β-lactams[c]

[a] Characteristical resistances that are partially used used for diagnostic purposes;
[b] Broad-spectrum β-lactamase TEM-1 frequently occurs in nonfermenters (P. aeruginosa, A. baumannii);
[c] Broad hydrolytic spectrum including carbapenems.

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Classification of ESBLs according to *Giske* et al., JAC 2009, 63, 1-4

Acquired β-lactamases with hydrolytic activity against extended-spectrum cephalosporins and/or carbapenems			
β-Lactamase classes	$ESBL_A$	$ESBL_M$	$ESBL_{CARBA}$
	High prevalent $ESBL_A$ CTX-M TEM-ESBLs SHV-ESBLs VEB PER	$ESBL_{M-C}$ (Plasmid-mediated AmpC) CMY FOX MIR MOX DHA LAT BIL ACT ACC	$ESBL_{CARBA-A}$ KPC GES-2, -4, -5, -6, -8 NMC SME IMI-1, -2
	Low prevalent $ESBL_A$ GES-1, -3, -7, -9 SFO-1 BES-1 BEL-1 TLA IBC CMT[a]	$ESBL_{M-D}$ (OXA-ESBL) OXA-10-group OXA-13-group OXA-2-group OXA-18 OXA-45	$ESBL_{CARBA-B}$ (MBL) IMP VIM SPM-1 GIM-1 SIM-1 AIM-1
			$ESBL_{CARBA-D}$ (OXA-carbapenemases) OXA-23-group OXA-24-group OXA-48[b] OXA-58-group
Operational definition	Non-susceptibility to extended-spectrum cephalosporins AND clavulanate synergy	Non-susceptibility to extended-spectrum cephalosporins AND phenotypic detection ($ESBL_{M-C}$) OR genotypic detection ($ESBL_{M-D}$)	Non-susceptibility to extended-spectrum cephalosporins and at least one carbapenem AND $ESBL_{CARBA}$ detected with phenotypic and/or genotypic methods

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ESBL: Extended Spectrum Beta-Lactamases

- Nowadays > 700 genes encoding ESBLs
- Confer resistance to:
  - Penicillins
  - 1e/2e generation cephalosporin (cefalexine ,cefapirine, cefoperazon, cefadroxil)
  - 3rd/4th generation cephalosporins (ceftiofur, cefquinome, cefovecine)
  - Monobactams (aztreonam)
  - Inhibited by clavulanic acid
- Mostly plasmid-mediated, incidentally located on the chromosome

AmpC

- Confer resistance to:
  - Penicillins
  - 1e/2e generation cephalosporin
  - 3rd but not to 4th generation cephalosporins
  - And resistant to cephamycins (cefoxitin)
  - Not inhibited by clavulanic acid
- Plasmid-mediated or chromosomally located

Chromosomal *ampC*

- Chromosomal *ampC* gene is present in almost all Enterobacteriaceae except for *Klebsiella* and *Proteus* spp
- But normally not producing enzymes in high levels
- For *E. coli*: high-level ampC production can occur due to:
  - Mutations in the promotor of the ampC gene (normally the promotor is degenerated)

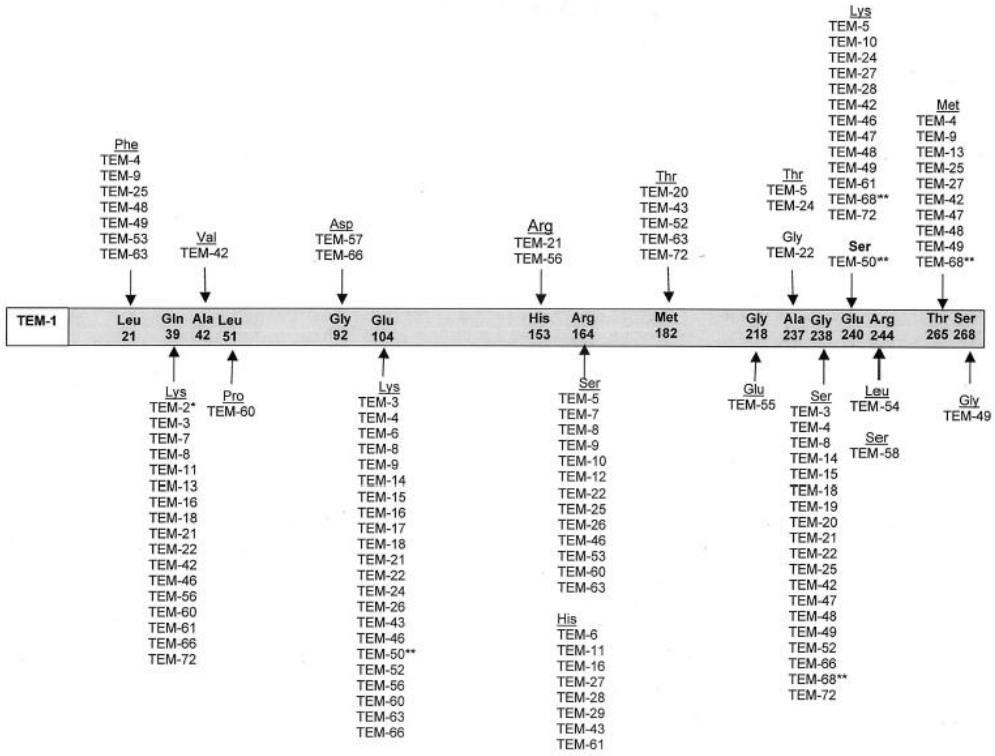
    (e.g. mutation at position -42 and -18, Caroff et al., 2000 and inserts, Siu et al., 2003)
- For other Enterobacteriaceae: high-level ampC production can occur due to:
  - mutations in *ampR* (regulating) or *ampD* amidase (inhibiting) gene.

ESBL and AmpC

- Most important ESBL and AmpC types at the moment
  - ESBL
    - TEM-group
    - SHV-group
    - CTX-M-group
  - AmpC
    - Plasmid-mediated: CMY
    - Chromosomal

TEM

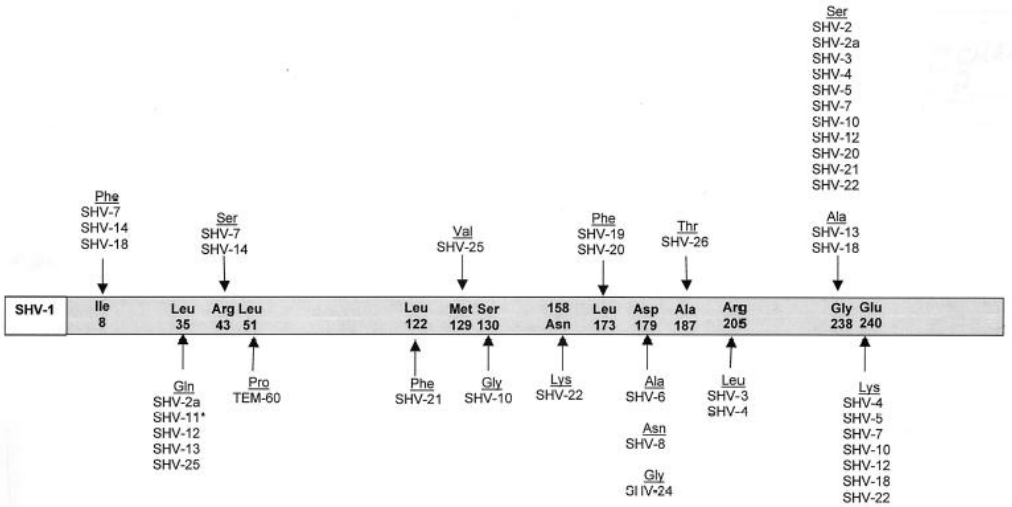
- TEM-1 found in 1960s isolated from a Greek patient named Temoneira
- ESBLs derived from mutations in TEM-1
- TEM-52 is most important ESBL-type (2be)
- Also broad-spectrum (2b) and inhibitor resistant TEM-types (2br)



Bradford, Clin. Microbiol.Rev. 2001, 14;933-51

SHV

- SHV: sulfhydryl reagent variable
- First ESBL: SHV-2 described in 1983 in Klebsiella ozaenae in Germany
- ESBLs derived from mutations in SHV-1
- SHV-2 and SHV-12 are the most important ESBL- types
- Within SHV-group also non-ESBL types (2b group) see www.lahey.org/studies



Bradford, Clin. Microbiol.Rev. 2001, 14;933-51

CTX-M

- CTX-M= active on cefotaxime first isolated in Munich
- Probably derived from Kluyvera sp. in the environment
- Occured since 2000, now most prevalent ESBL-types
- Divided in 5 groups (see also www.lahey.org/studies)
  - **CTX-M-1 group** (most important CTX-M-1, CTX-M-15)
  - **CTX-M-2 group** (most important CTX-M-2)
  - CTX-M-8 group
  - **CTX-M-9 group** (most important CTX-M-9 and CTX-M-14)
  - CTX-M-25 group

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The CTX-M Conundrum: Dissemination of Plasmids and *Escherichia coli* Clones

Umaer Naseer[1] and Arnfinn Sundsfjord[1,2]

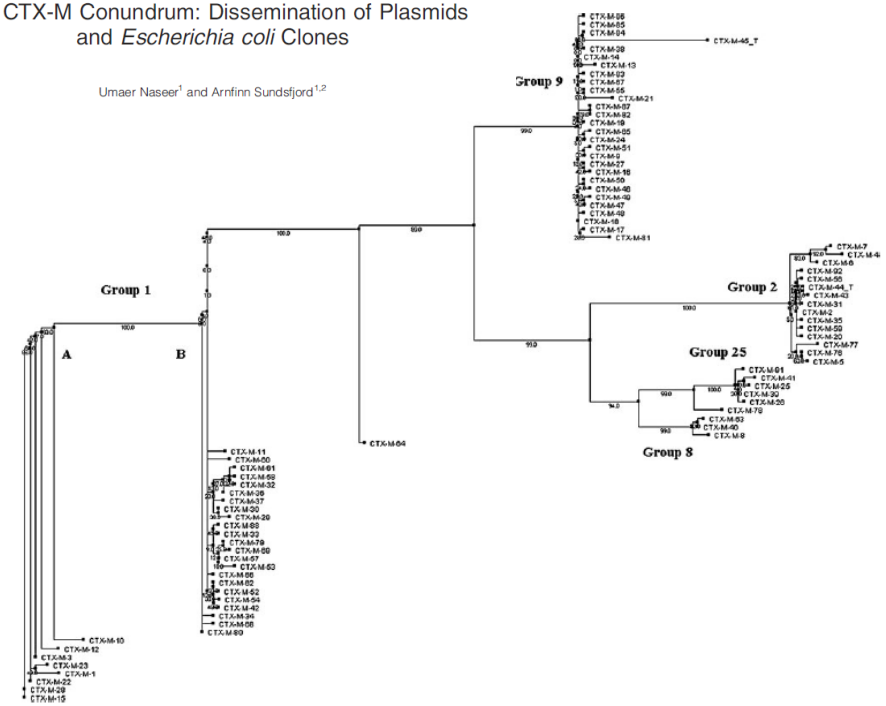


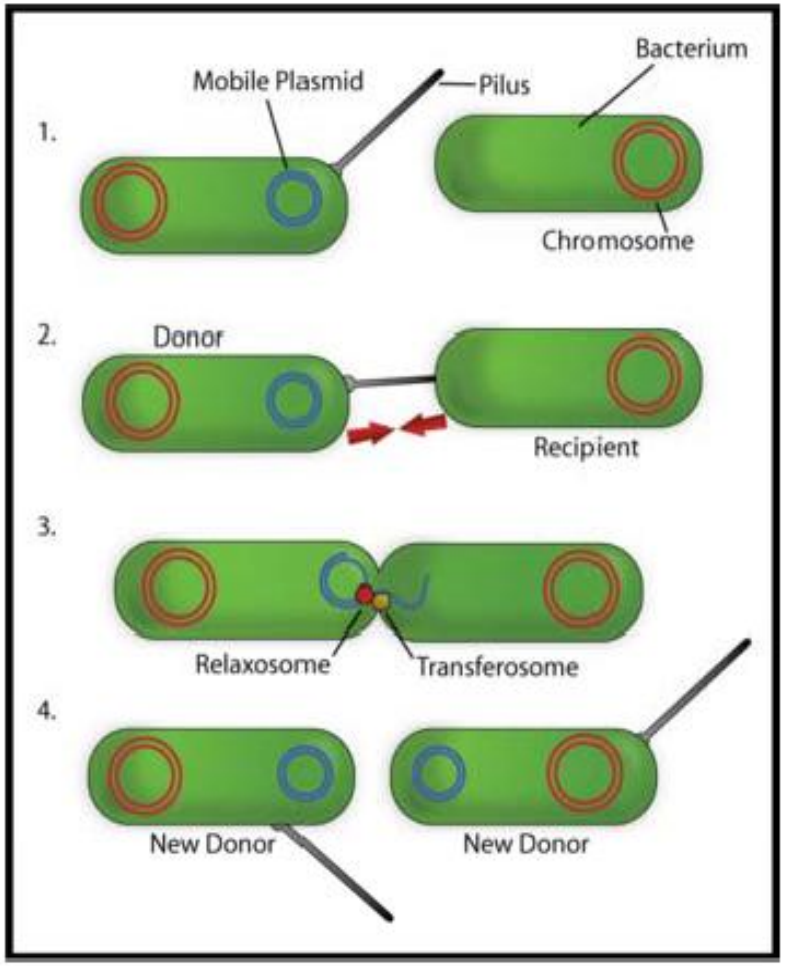
FIG. 1. Phylogenetic maximum likelihood (PhyML 3.0) Phylogram of 88 CTX-M amino acid sequences acquired from NCBI GenBank database. The tree distance phylogeny reconstruction is according to a BioNJ algorithm, with an LG amino acid substitution model and 100 bootstrapped data sets. The Phylogram displays five major CTX-M clusters previously identified and labeled as groups 1, 2, 8, 9, and 25. Additionally, the Phylogram shows that Group 1 genotypes have diversified into two subgroups based upon their amino acid substitutions (A and B) (www.atgc-montpellier.fr/phyml/).[58]

CMY

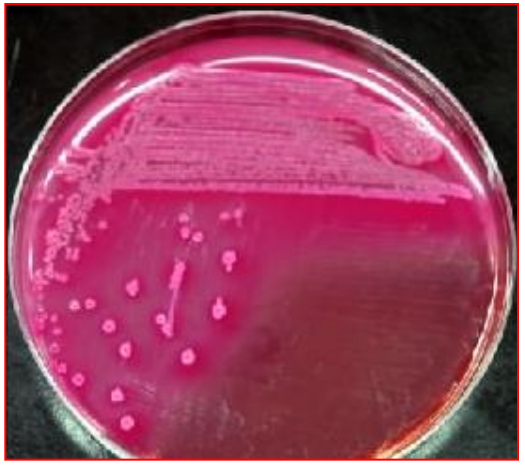
- Derived from chromosomal *ampC* genes from *Citrobacter freundii* or *Enterobacter cloacae*
- First plasmid-mediated ampC gene was found in 1989: CMY-1 in *Klebsiella pneumoniae*
- Most important one now is CMY-2

Why are ESBLs and AmpCs so important?

- 3rd and 4th generation cephalosporins are listed as critically important for human health (WHO)
- ESBL/AmpC genes mostly located on plasmids
- Horizontal transfer of genes between similar bacterial species (*E. coli* -> *E. coli*) but also between other bacterial species (*E. coli* -> *Salmonella*)
- Plasmid can contain multiple resistance genes→ multi-resistence (less treatment options)



Detection of ESBL en AmpC



- Non-selective
- Selective

Non-selective detection (further info: EFSA panel on BIOHAZ)

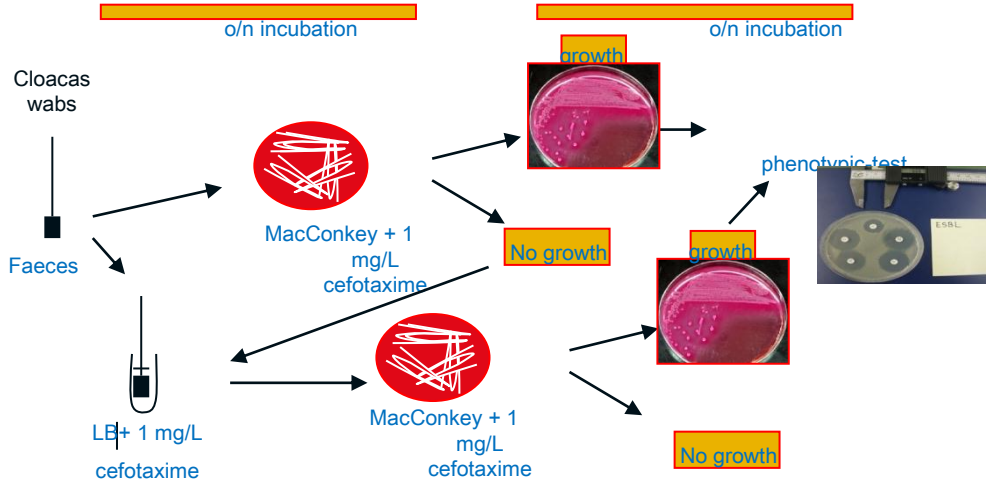
- Resistance to indicator 3rd generation cephalosporins
  - Cefotaxime and ceftazidime for detection of organisms with plasmid mediated ESBL genes
  - EUCAST Epidemiological cut-off values (www.eucast.org)

MIC	E.coli	Salmonella
Cefotaxime	$\leq$ 0.25 mg/L	$\leq$ 0.5 mg/L
Ceftazidime	$\leq$ 0.5 mg/L	$\leq$ 2 mg/L

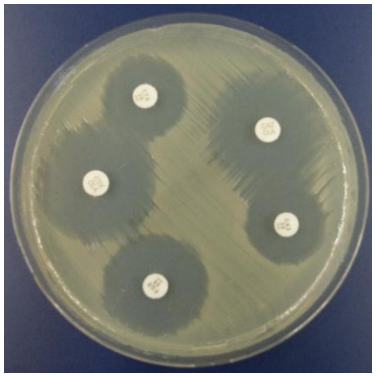
  - Do not use Cefpodoxime for screening
- Confirmation by phenotypic tests

Selective detection

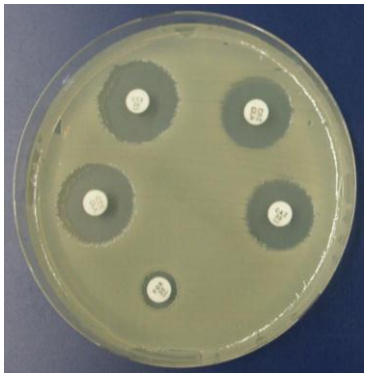
- Pre-enrichment with broth containing 1 mg/L cefotaxime or ceftriaxone (non-quantitative)
- Chromogenic agar (e.g. MacConkey) with 1 mg/L cefotaxime of ceftriaxone
- Confirmation of isolates by phenotypic tests



Phenotypic tests

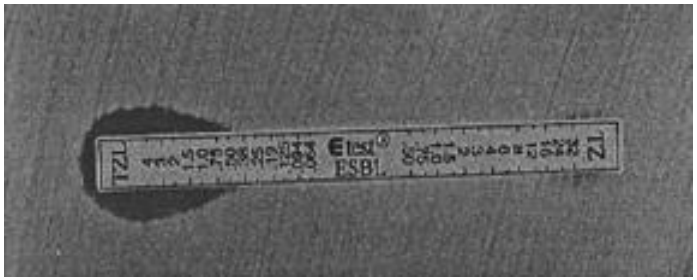


ESBL



AmpC

- Combination disk tests (CLSI)
  - Cefotaxime (≤22mm) with/without clavulanic acid → zone with clavulanic acid ≥ 5 mm larger
  - Ceftazidime(≤27mm) with/without clavulanic acid → zone with clavulanic acid ≥ 5 mm larger
  - Cefoxitin (≤ 18 mm is indicative for AmpC-production)
- E-test
  - ≥ 8 fold reduction with clavulanic acid



Molecular detection of ESBL/AmpC genes

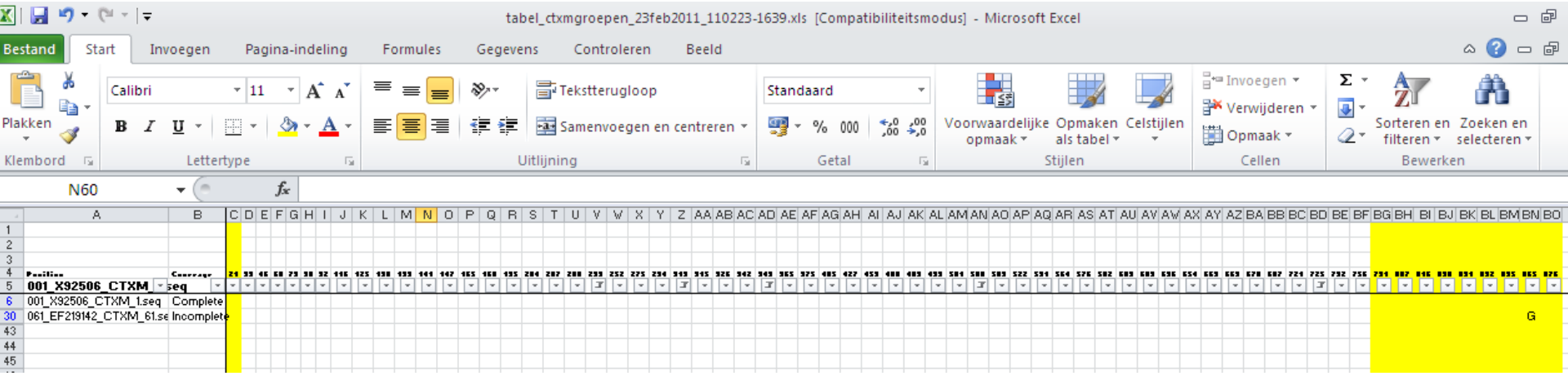
- Divide enzymes in groups by:
  - Array (Clondiag or Checkpoints)
  - Isoelectric focussing (not very sensitive for detection of all beta-lactamases?, *Sharma et al.*, 2010)
  - (Multi-)plex PCRs
- Confirmation of gene by:
  - PCR/sequencing

PCR/sequence

- Aim to have primers outside the gene to sequence the whole gene
- In this course:
  - TEM: all types detected
  - CTX-M-1: no distinction between CTX-M-1 and CTX-M-61
  - CTX-M-9: all types detected
  - CMY: no distinction between CMY-2 and CMY-22

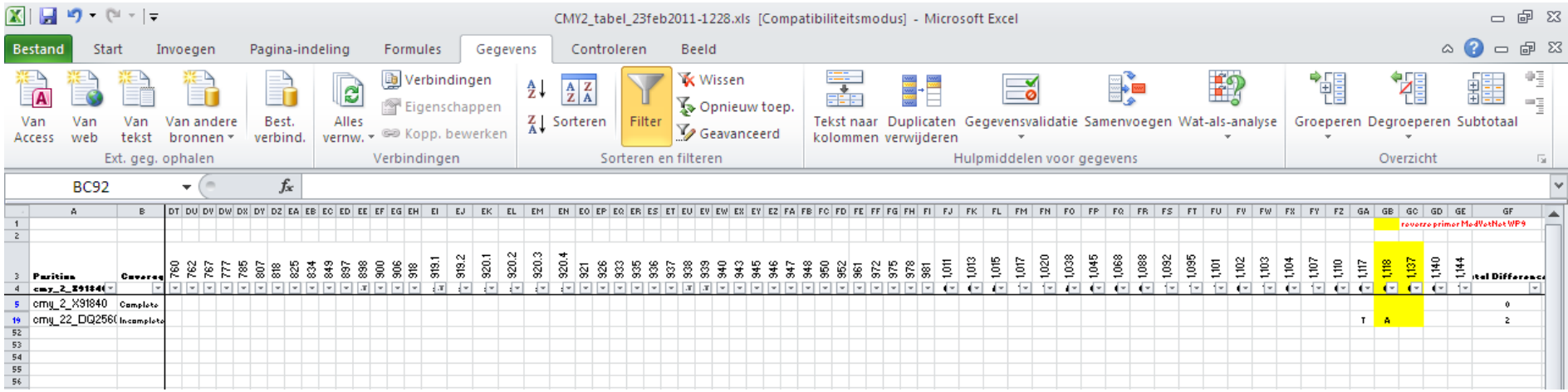
CTX-M-1 and CTX-M-61

Reverse primer: 862-842,mutation at position 865



- Other option:
  - primers described by Carattoli et al., 2008, *J. Clin.Microbiol 46:103-8*

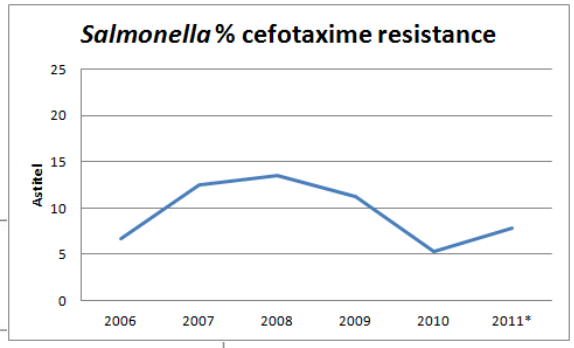
CMY-2 and CMY-22
Reverse primer: 1138-1118
Mutation at position 1118

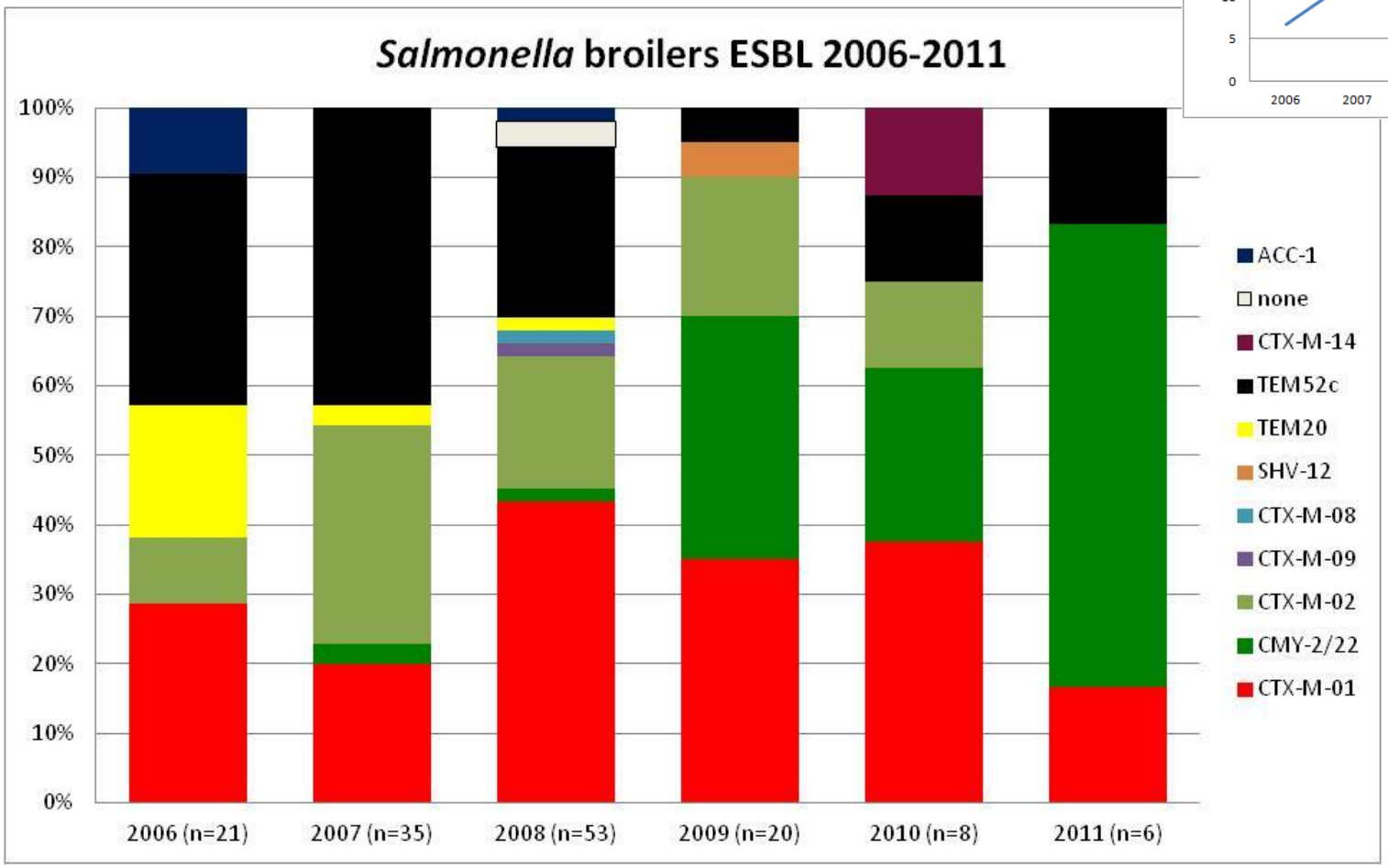


Further characterisation

- Plasmid typing (tomorrow)

Results monitoring program – ESBL/AmpC genes – *Salmonella* in broilers in NL





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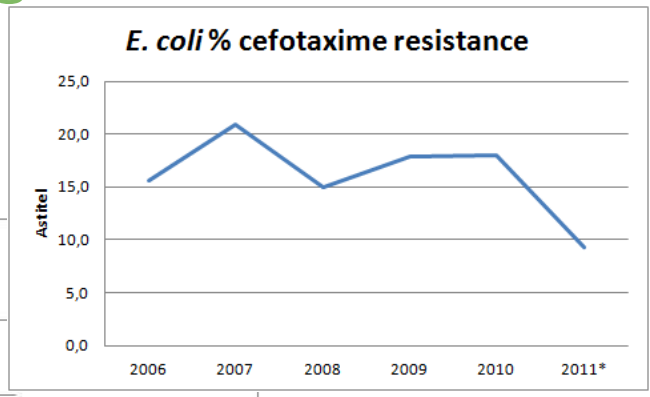

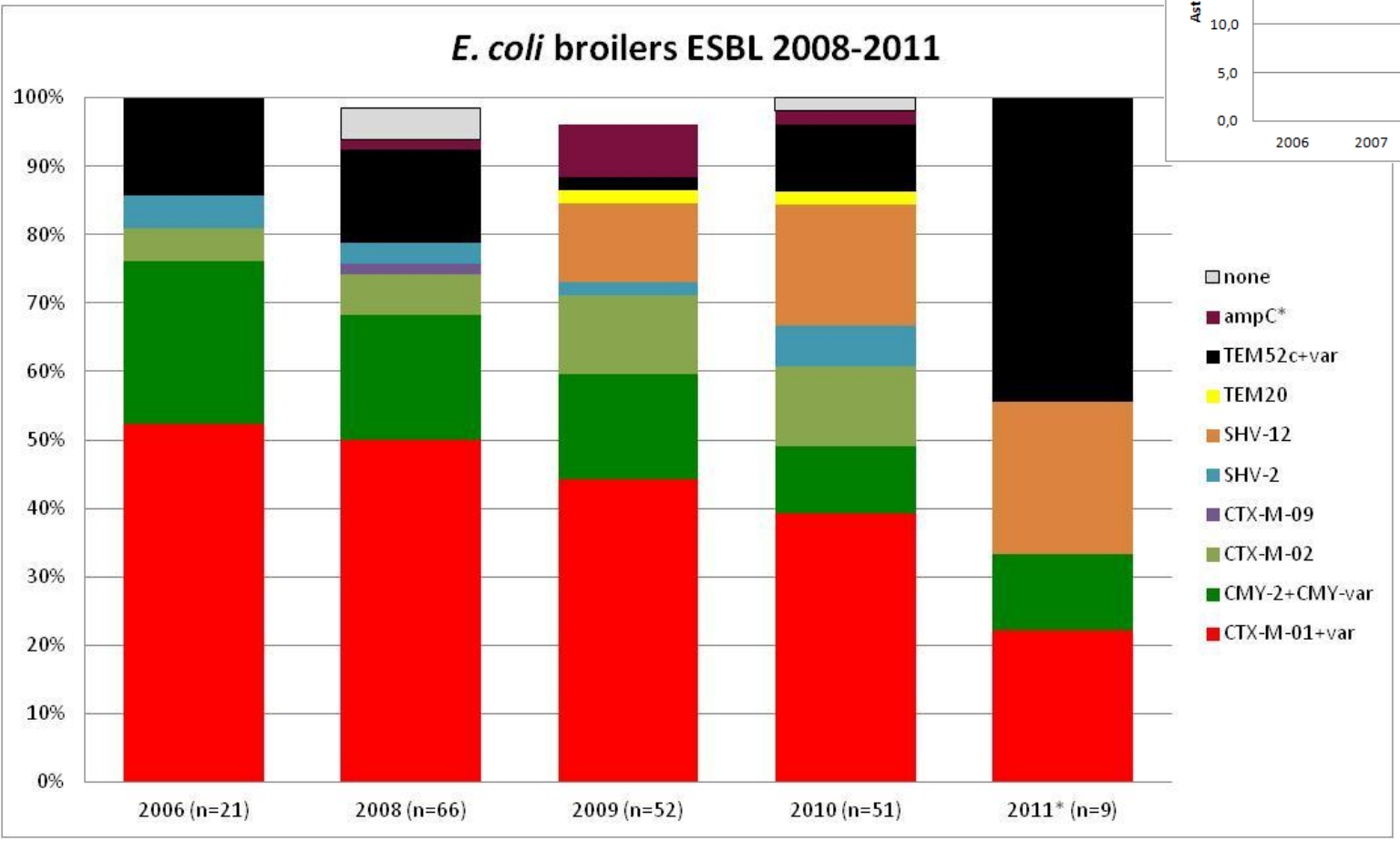

Risk to human health

ORIGINAL ARTICLE 10.1111/j.1469-0691.2011.03497.x

Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains

M. A. Leverstein-van Hall[1,2], C. M. Dierikx[3], J. Cohen Stuart[1], G. M. Voets[1], M. P. van den Munckhof[1], A. van Essen-Zandbergen[3], T. Platteel[1,4], A. C. Fluit[1], N. van de Sande-Bruinsma[2], J. Scharinga[1], M. J. M. Bonten[1,5] and D. J. Mevius[3,6]; on behalf of the national ESBL surveillance group*

1) *Department of Medical Microbiology, University Medical Centre Utrecht, Utrecht,* 2) *Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven,* 3) *Department of Bacteriology and TSEs, Central Veterinary Institute of Wageningen UR, Lelystad,* 4) *SALTRO, Primary Health Care Laboratory, Utrecht,* 5) *Julius Centre for Health Sciences and Primary Care, University Medical Centre, Utrecht and* 6) *Department of Infectious Diseases & Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands*

Level of genetic typing	% of human isolates with poultry associated genetic element[a]
ESBL genes ($bla_{CTX\text{-}M\text{-}1}$, $bla_{TEM\text{-}52}$, $bla_{SHV\text{-}12}$, $bla_{SHV\text{-}2}$ and $bla_{CTX\text{-}M\text{-}2}$)	35% (see Table 1)
$bla_{CTX\text{-}M\text{-}1}$ and $bla_{TEM\text{-}52}$ genes	30% (23.7% $bla_{CTX\text{-}M\text{-}1}$; 6.2% $bla_{TEM\text{-}52}$)
$bla_{CTX\text{-}M\text{-}1}$ and $bla_{TEM\text{-}52}$ genes on IncI1 plasmid	20% (14.2% $bla_{CTX\text{-}M\text{-}1}$; 6.2% $bla_{TEM\text{-}52}$)
$bla_{CTX\text{-}M\text{-}1}$ and $bla_{TEM\text{-}52}$ genes on IncI plasmid belonging to complex CC7 or CC3 and CC5 resp.	19% (12.6% $bla_{CTX\text{-}M\text{-}1}$; 6.2% $bla_{TEM\text{-}52}$)
$bla_{CTX\text{-}M\text{-}1}$ and $bla_{TEM\text{-}52}$ genes on IncI plasmid belonging to complex CC7 or CC3 and CC5 resp. in a poultry-associated MLST strain (ST10, ST58 or ST117)	11% (9.5% $bla_{CTX\text{-}M\text{-}1}$; 2.0% $bla_{TEM\text{-}52}$)

ESBLs in chicken meat, human rectal swabs, human blood cultures

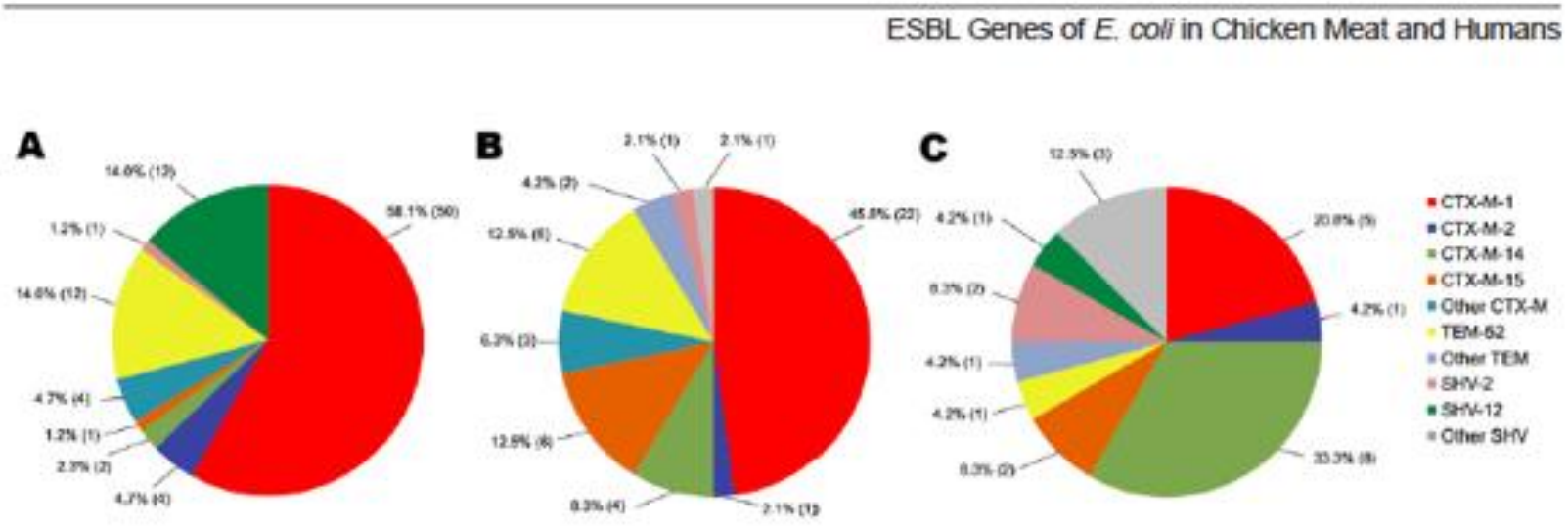

Figure 1. Distribution of extended-spectrum β-lactamase genes in chicken meat (A), human rectal swabs (B), and human blood cultures (C), the Netherlands. Values in parentheses are no. positive.

Overdevest I et al. Emerg Infect Dis. 2011 Jul. http://www.cdc.gov/EID/content/17/7/1216.htm

Thank you for your attention!

- Questions????