Zoonotic perspective of ESBLs in Animals

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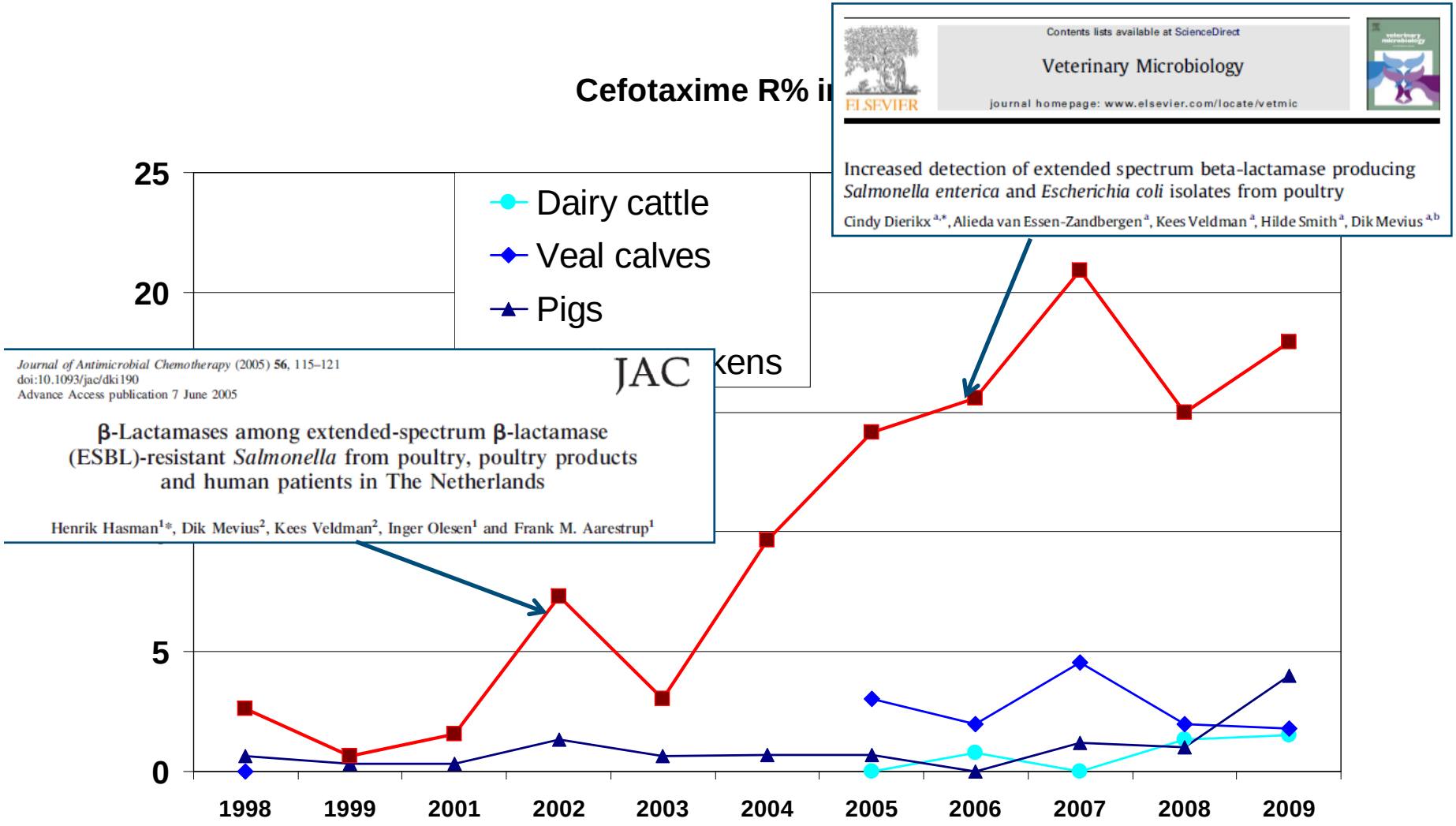
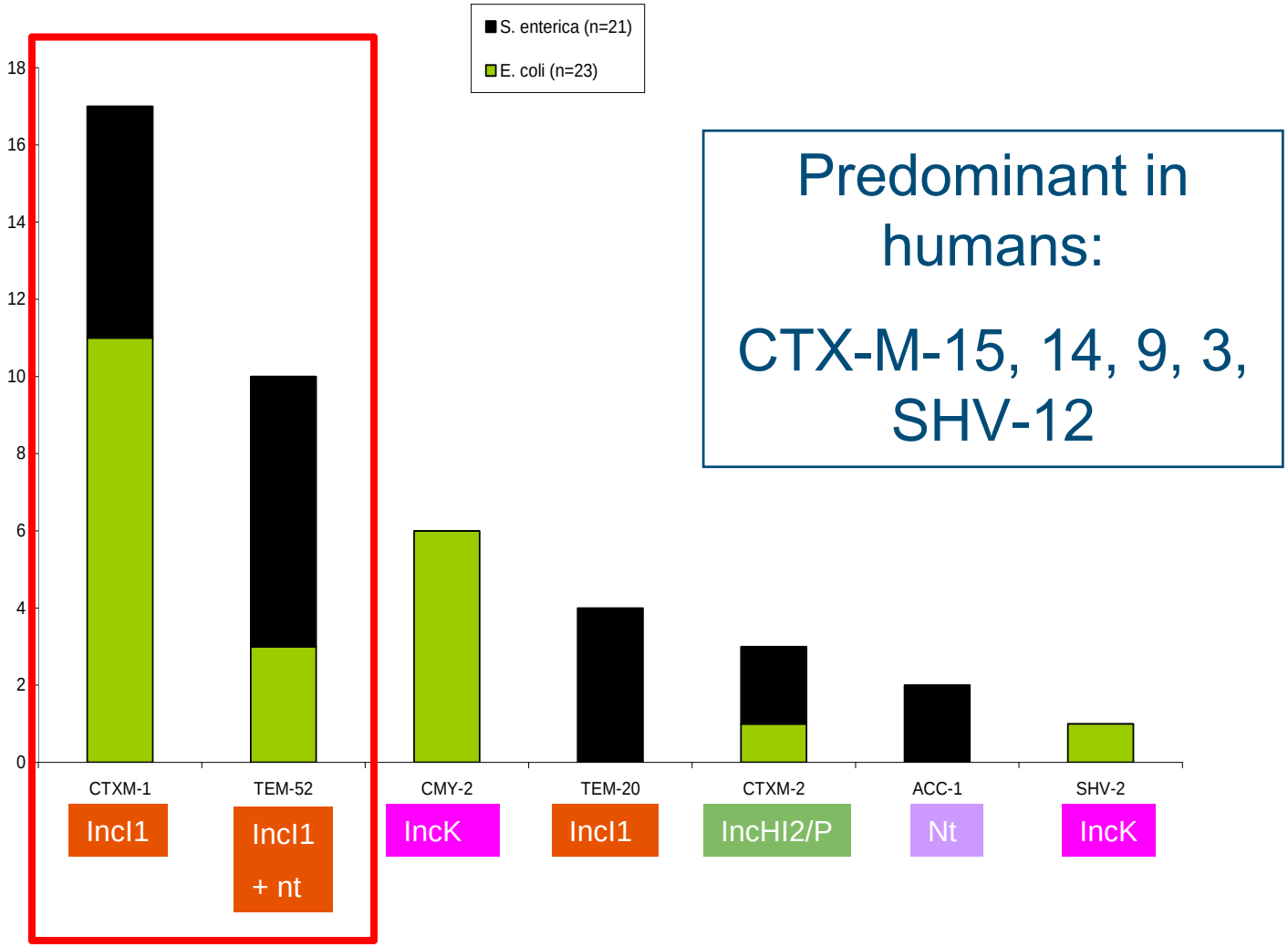
Cefotaxime resistance in E. coli (MARAN)



ESBL-genes and plasmids in Broiler isolates

(Dierikx et al. 2010)



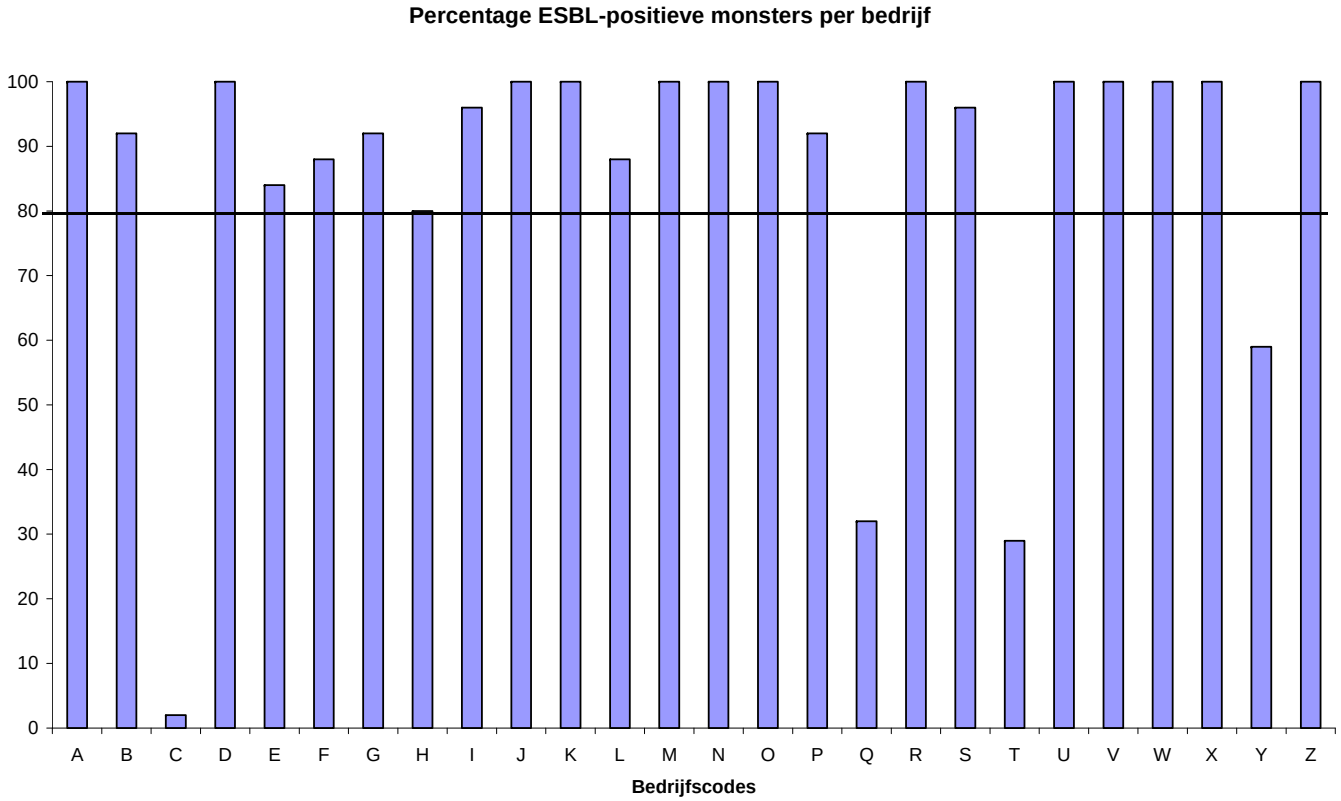
Predominant in humans:

CTX-M-15, 14, 9, 3, SHV-12

Prevalence of ESBLs on Dutch broiler farms

(Dierikx et al. NvMM)

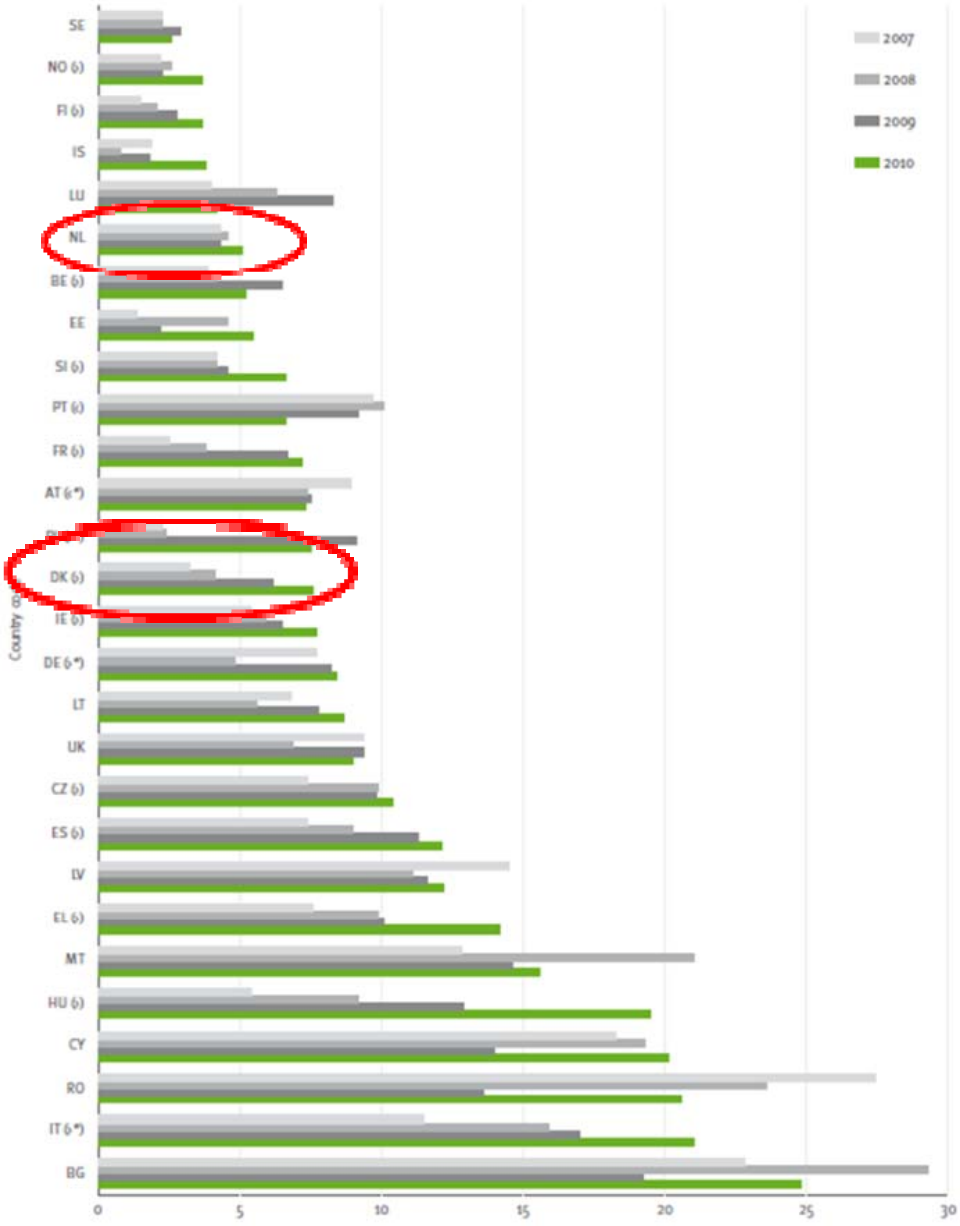
- 100 % of the farms: ESBL-positive
- On 85 % (22/26) within-farm prevalence ≥ 80%



ESBLs in humans in NL

(EARRS-Net 2010)

Figure 5.18: *Escherichia coli*: trends of resistance to third-generation cephalosporins by country, 2007–2010

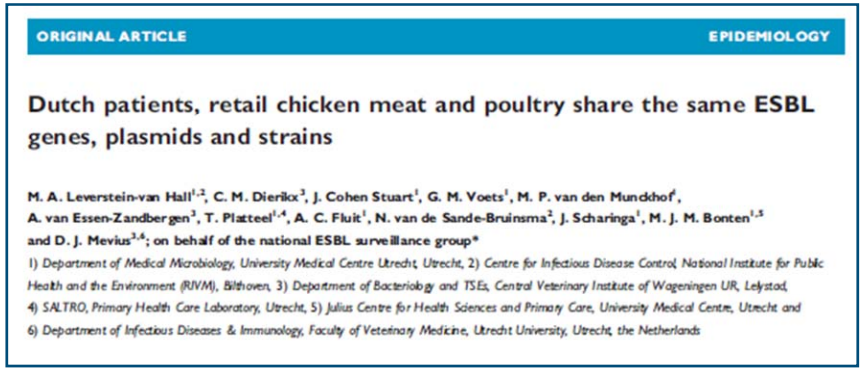


Which types of ESBLs in humans in NL?

- National ESBL reference study (RIVM, UMCU, CVI) analysing 692 human clinical isolates from 29 diagnostic laboratories (Leverstein et al, NVMM 2010)
  - 75% E. coli
    - App. half was CTX-M-15 (≈ pandemic variant in humans – no relation with farm animals!!)
    - (CTX-M-9/14, 2, 3; SHV-12)
    - Partially (30%) either CTX-M-1 or TEM-52
      (Paltansing LUMC, 24% CTX-M-1 P054)
      (Al Naimi et al. JCM 2006, 18% CTX-M-1)

Potential relation with poultry!!!

Association with human ESBLs



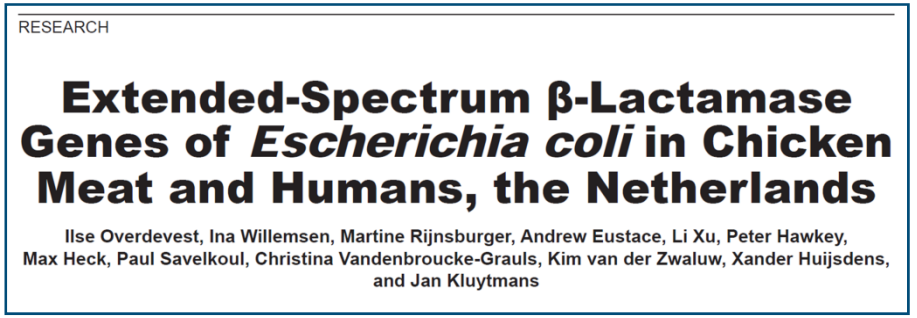
ORIGINAL ARTICLE EPIDEMIOLOGY

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[4], 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

CMI, 2011



RESEARCH

Extended-Spectrum β-Lactamase Genes of *Escherichia coli* in Chicken Meat and Humans, the Netherlands

Ilse Overdevest, Ina Willemsen, Martine Rijnsburger, Andrew Eustace, Li Xu, Peter Hawkey, Max Heck, Paul Savelkoul, Christina Vandenbroucke-Grauls, Kim van der Zwaluw, Xander Huijsdens, and Jan Kluytmans

EID, 2011

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

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journal homepage: www.elsevier.com/locate/ijfoodmicro

Short Communication

Comparison of ESBL contamination in organic and conventional retail chicken meat

James Cohen Stuart [a,*], Thijs van den Munckhof [a], Guido Voets [a], Jelle Scharringa [a], Ad Fluit [a], Maurine Leverstein-Van Hall [a,b]

[a] Department of Medical Microbiology, University Medical Center Utrecht, The Netherlands
[b] Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

84 – 100% of poultry meat pos for PA-ESBLs
(CTX-M-1, TEM-52)

- Conclusion:
  - Yes an animal attribution is apparent
  - Poultry meat was considered to be the most likely source

SafefoodERA study

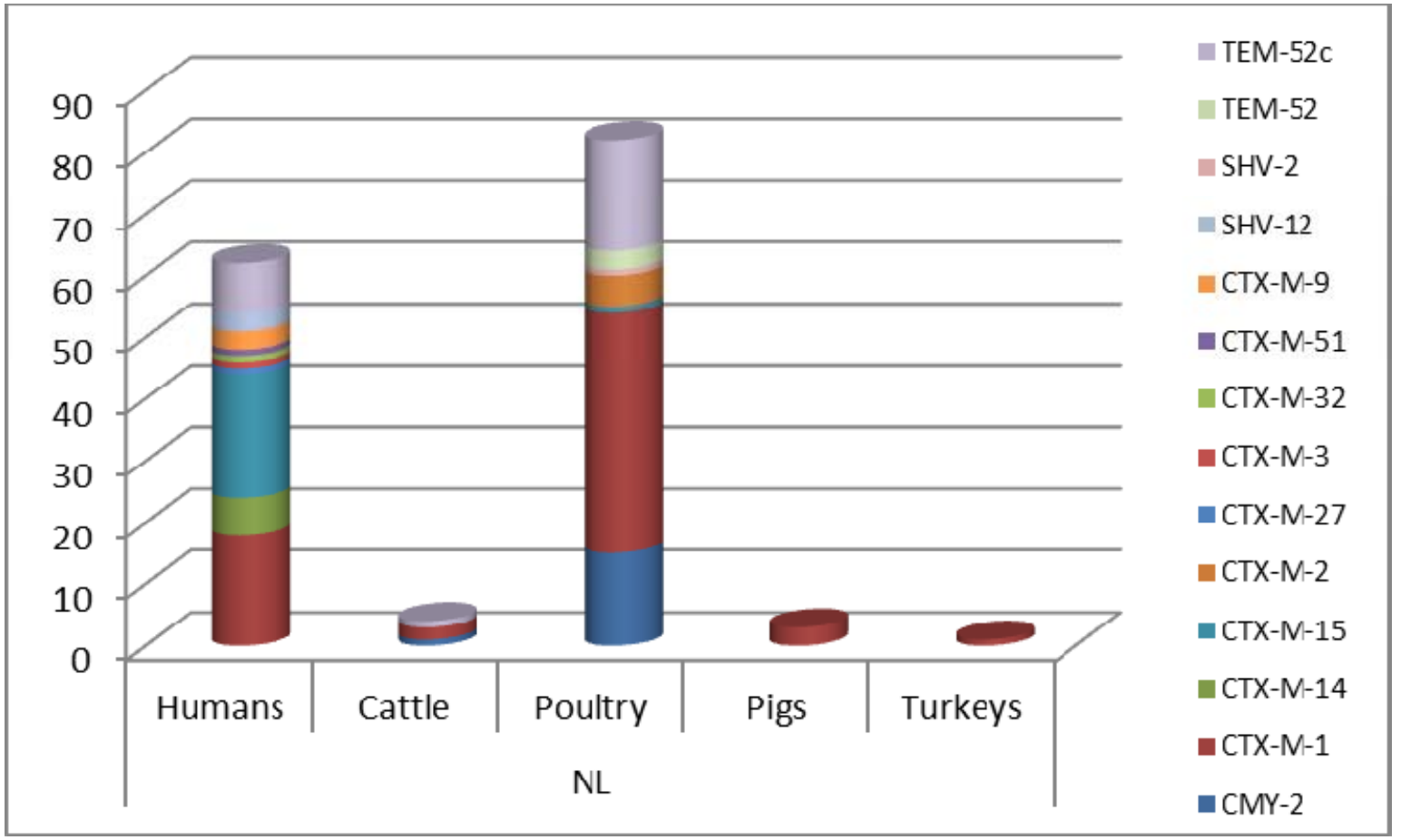
- **Title: The Role of Commensal Microflora in the transmission of ESBLs**
- **Aim:** to compare the characteristics of pathogenic and commensal *E. coli* and the food-borne pathogen *Salmonella* harbouring ESBLs in the community, foods for human consumption, health care and animal sources.
- **Partners:**
  - **UK: AHVLA, HPA**
  - **G: FLI, BfR**
  - **NL: CVI**

Activities within the project

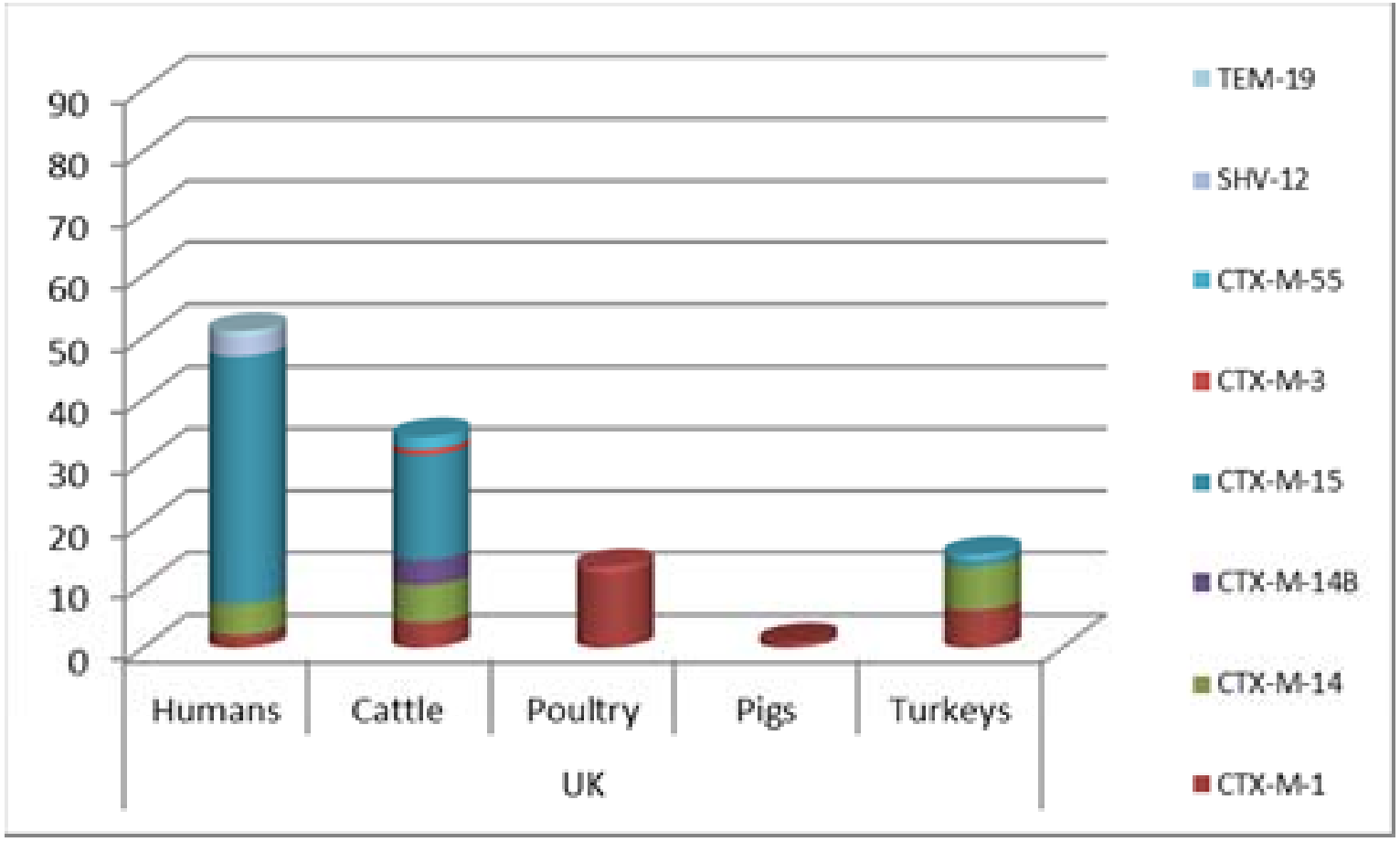
- Build a large strain collection of ESBL-suspected E. coli (Salmonella) from humans, animals and food
- Selected app 400 recent isolates (NL 2009)
  (368 ESBL-pve E. coli, 50 neg controls)

  - Characterised plasmid mediated ESBL/AmpC-genes
    - Microarray, PCR-sequencing
  - Plasmid isolation and characterisation
  - Phylogrouping, Genotyping (MLST)

ESBLs in NL


90
80
70
60
50
40
30
20
10
0
Humans
Cattle
Poultry
Pigs
Turkeys
NL
TEM-52c
TEM-52
SHV-2
SHV-12
CTX-M-9
CTX-M-51
CTX-M-32
CTX-M-3
CTX-M-27
CTX-M-2
CTX-M-15
CTX-M-14
CTX-M-1
CMY-2

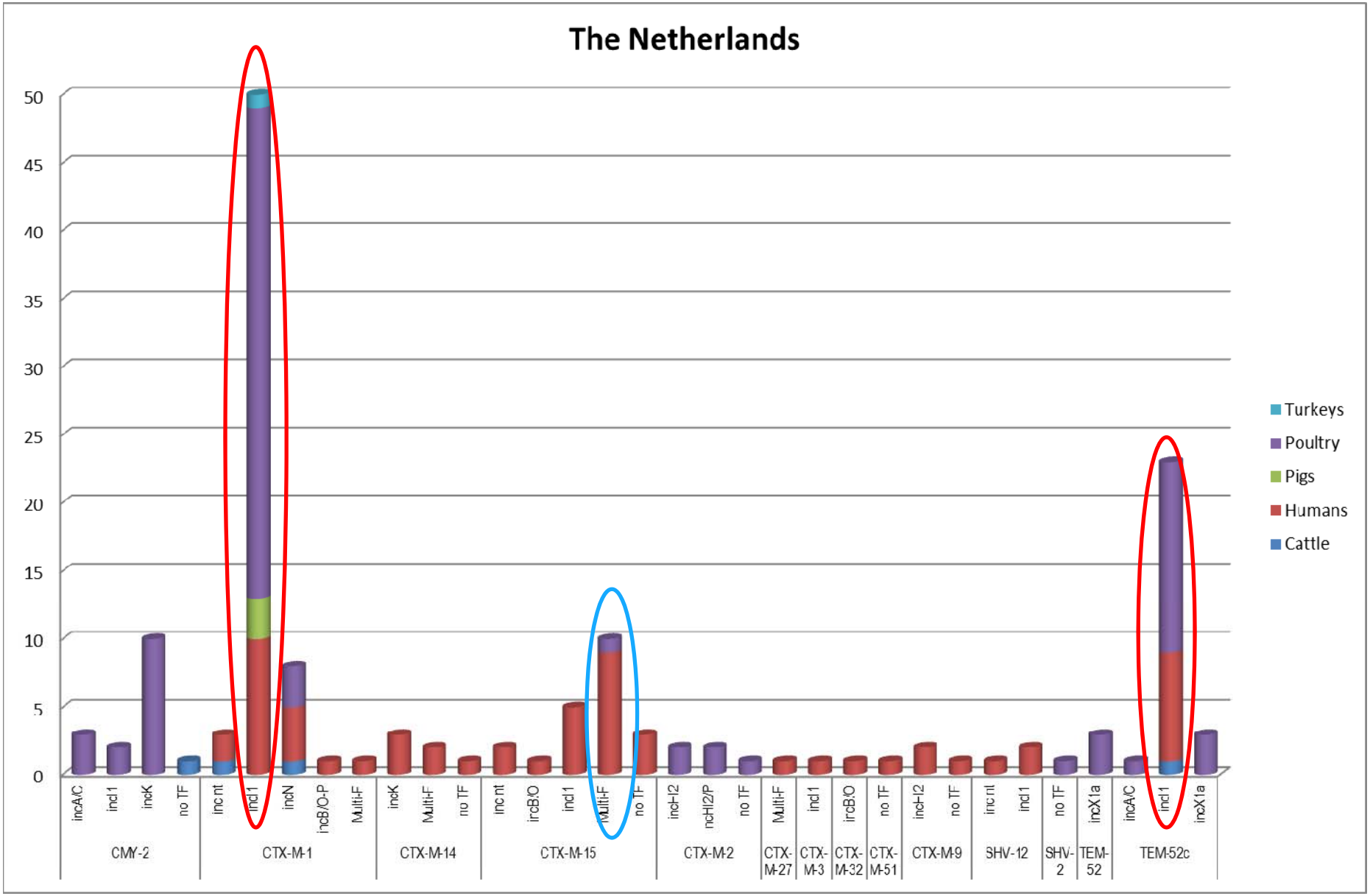
ESBLs in UK

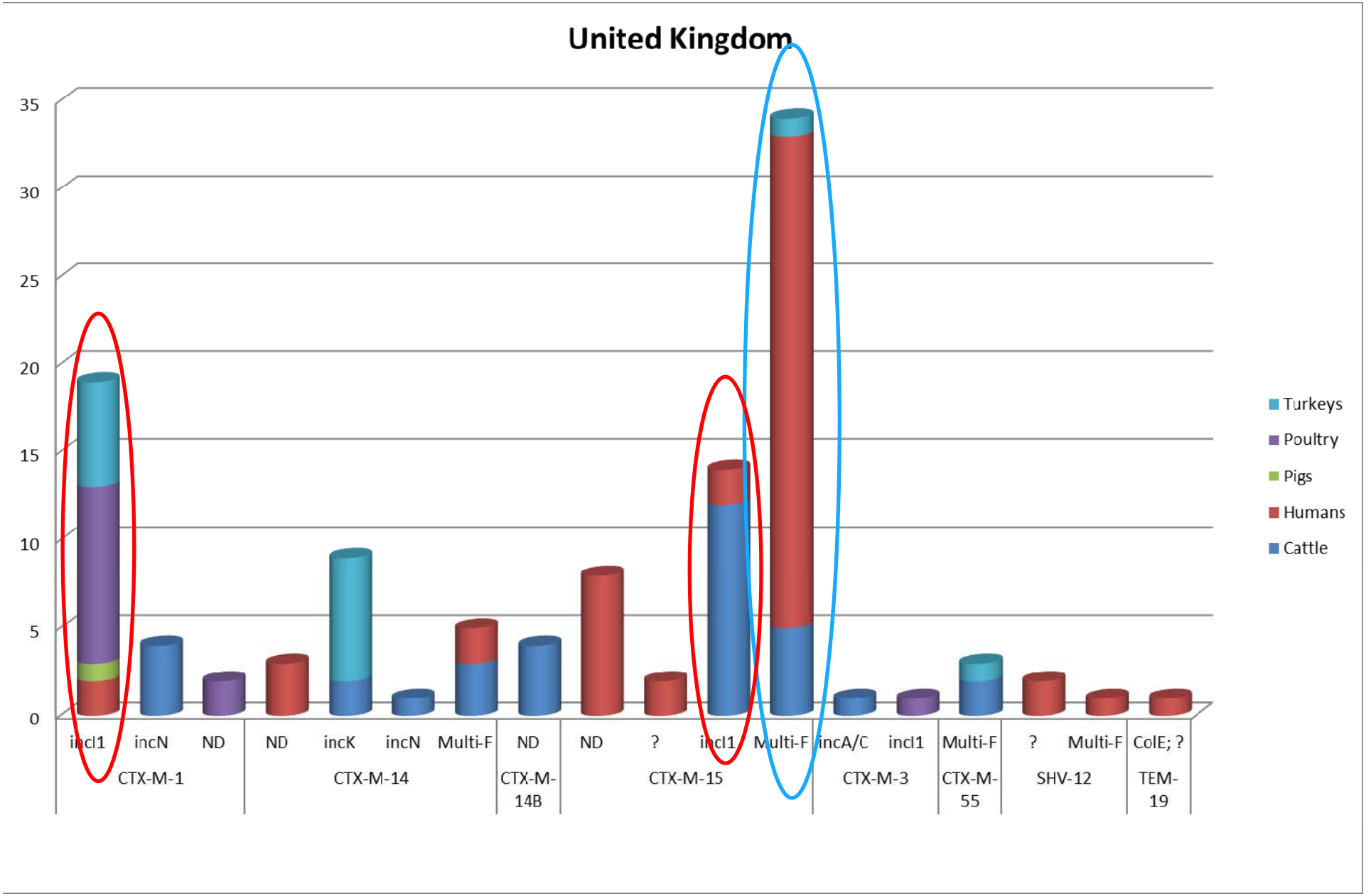

90
80
70
60
50
40
30
20
10
0
Humans
Cattle
Poultry
Pigs
Turkeys
UK
TEM-19
SHV-12
CTX-M-55
CTX-M-3
CTX-M-15
CTX-M-14B
CTX-M-14
CTX-M-1

Plasmid identification and characterisation

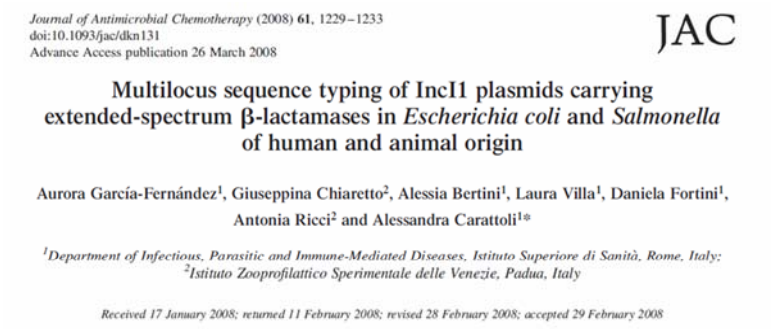
- Electroporation to competent cells
- Transformants isolated on MacConkey agar with 1 mg/L cefotaxime
  - Result was isolates harbouring a single plasmid/ESBL
  - PCR-based replicon typing (Caratttoli et al.)
  - Size determinations by S1-nuclease PFGE
  - PCR/hybridisation to confirm the presence of the ESBL

	Plasmids															
ESBL/AmpC group	**Enzyme**	**?**	**ColE; ?**	**inc nt**	**incA/C**	**incB/O**	**incHI2**	**incHI2-P**	**incI1**	**incK**	**incN**	**incB/O-P**	**incX1a**	**Multi-F**	**no TF**	**Grand Total**
CTX-M gr 1	CTX-M-1	3		3		5	4		87		22	2		5		134
	CTX-M-3				1				3							4
	CTX-M-15	3		2		1			20					49	3	87
	CTX-M-32					1										1
	CTX-M-55													3		3
CTX-M gr 2	CTX-M-2						3	3							1	7
CTX-M gr 9	CTX-M-9						2								1	3
	CTX-M-14									13	1			7	1	25
	CTX-M-14B															4
	CTX-M-27													1		1
	CTX-M-51														1	1
TEM	TEM-19		1													1
	TEM-52				1				24				6			31
SHV	SHV-2														1	1
	SHV-12	2		1					2					1		6
AmpC	CMY-2				3				4	17					1	25
	?													1		7
	(blank)									1					3	15
Grand Total		8	1	6	5	7	9	3	140	31	23	2	6	67	12	356


The Netherlands
50
45
40
35
30
25
20
15
10
5
0
Turkeys
Poultry
Pigs
Humans
Cattle
incA/C
incI1
incK
no TF
CMY-2
inc nt
incI1
incN
incB/O-P
Multi-F
CTX-M-1
incK
Multi-F
no TF
CTX-M-14
inc nt
incB/O
incI1
Multi-F
no TF
CTX-M-15
incHI2
incHI2/P
no TF
CTX-M-2
Multi-F
CTX-M-27
incI1
CTX-M-3
incB/O
CTX-M-32
no TF
CTX-M-51
incHI2
no TF
CTX-M-9
inc nt
incI1
SHV-12
no TF
SHV-2
incX1a
TEM-52
incA/C
incI1
incX1a
TEM-52c


United Kingdom
35
30
25
20
15
10
5
0
incI1
incN
ND
ND
incK
incN
Multi-F
ND
ND
?
incI1
Multi-F
incA/C
incI1
Multi-F
?
Multi-F
ColE; ?
CTX-M-1
CTX-M-14
CTX-M-14B
CTX-M-15
CTX-M-3
CTX-M-55
SHV-12
TEM-19
Turkeys
Poultry
Pigs
Humans
Cattle

Plasmid Multi Locus Sequence Typing (pMLST)



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doi:10.1093/jac/dkn131
Advance Access publication 26 March 2008

JAC

Multilocus sequence typing of IncI1 plasmids carrying extended-spectrum β-lactamases in *Escherichia coli* and *Salmonella* of human and animal origin

Aurora García-Fernández[1], Giuseppina Chiaretto[2], Alessia Bertini[1], Laura Villa[1], Daniela Fortini[1], Antonia Ricci[2] and Alessandra Carattoli[1]*

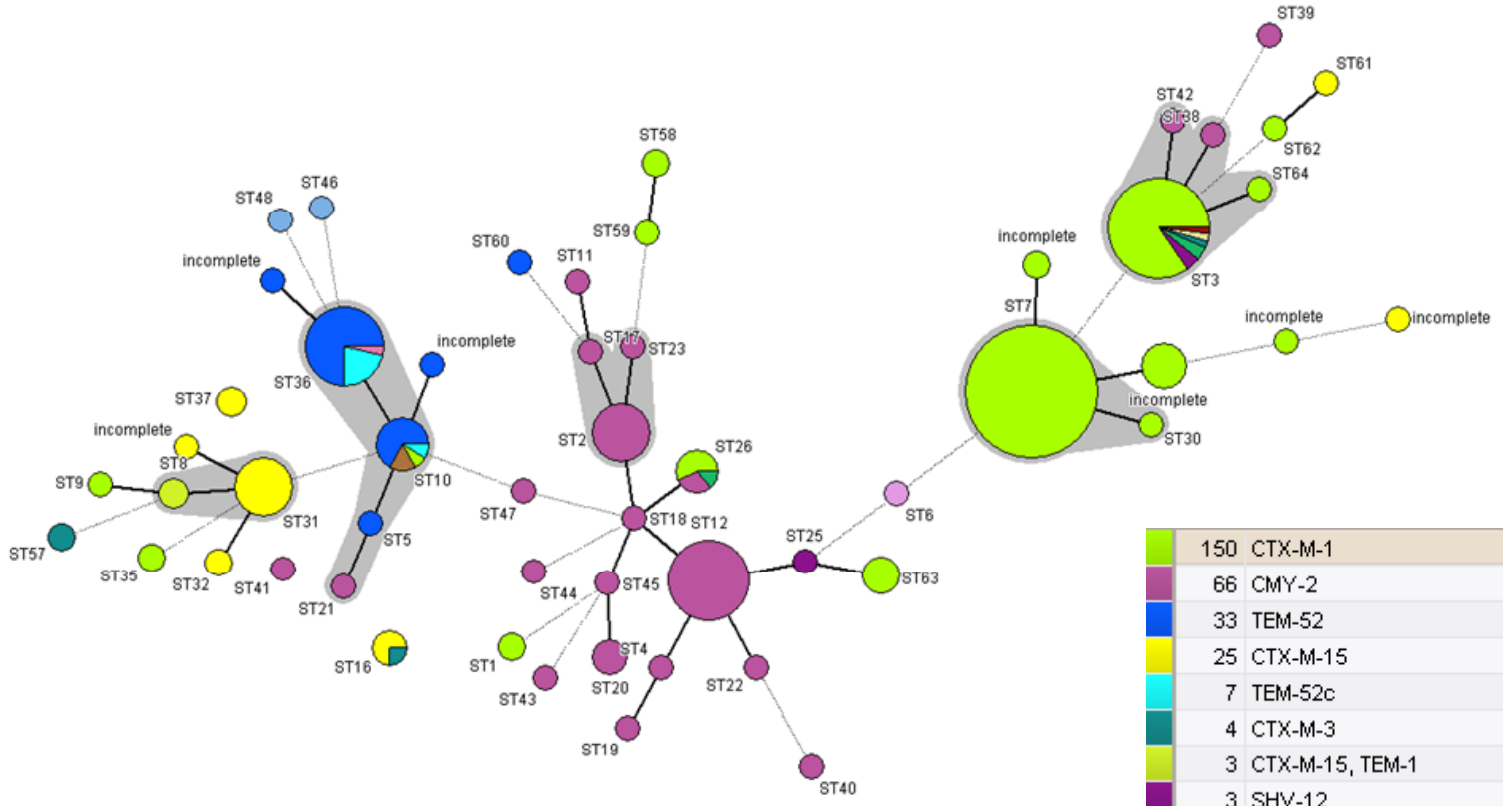
[1]*Department of Infectious, Parasitic and Immune-Mediated Diseases, Istituto Superiore di Sanità, Rome, Italy;* [2]*Istituto Zooprofilattico Sperimentale delle Venezie, Padua, Italy*

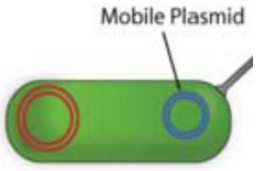
Received 17 January 2008; returned 11 February 2008; revised 28 February 2008; accepted 29 February 2008

Replicon type	rep I1	ardA	trbA	sogS	pilL	ST	clonal complex
incI1	2	1	5	4	2	7	CC-7
incI1	3	2	6	13	8	57	
incI1	2	1	5	4	2	7	CC-7
incI1	2	1	4	1	2	3	CC-3
incI1	1	4	2	9	3	36	CC-5
incI1	2	1	5	4	2	7	CC-7
incI1	1	8	12	3	7	37	
incI1	1	4	2	3	3	10	CC-5
incI1	1	4	2	9	3	36	CC-5
incI1	3	4	6	3	3	31	CC-31

302 IncI1-plasmids associated with ESBLs

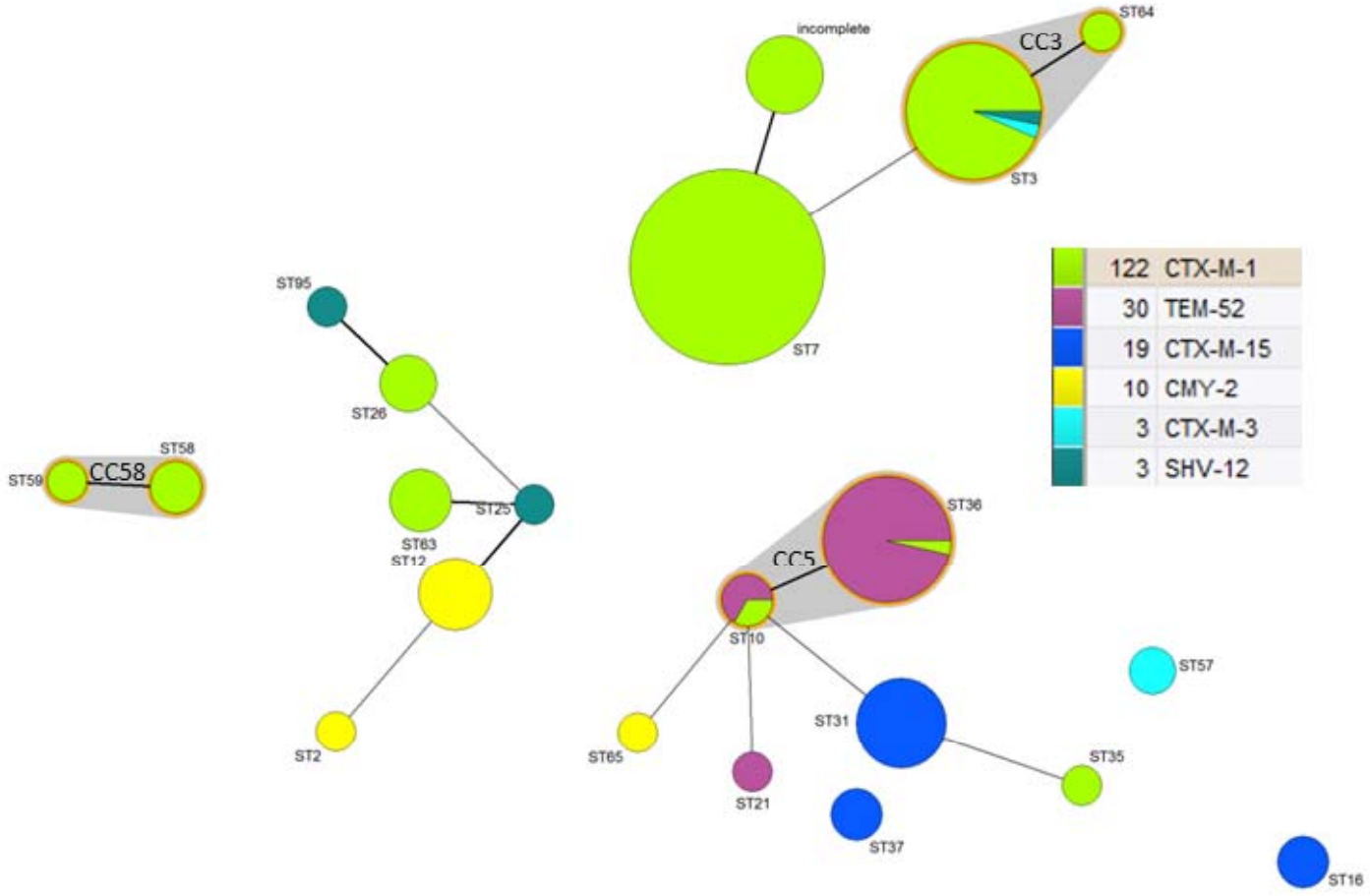
(www.pubmlst.org/plasmid)



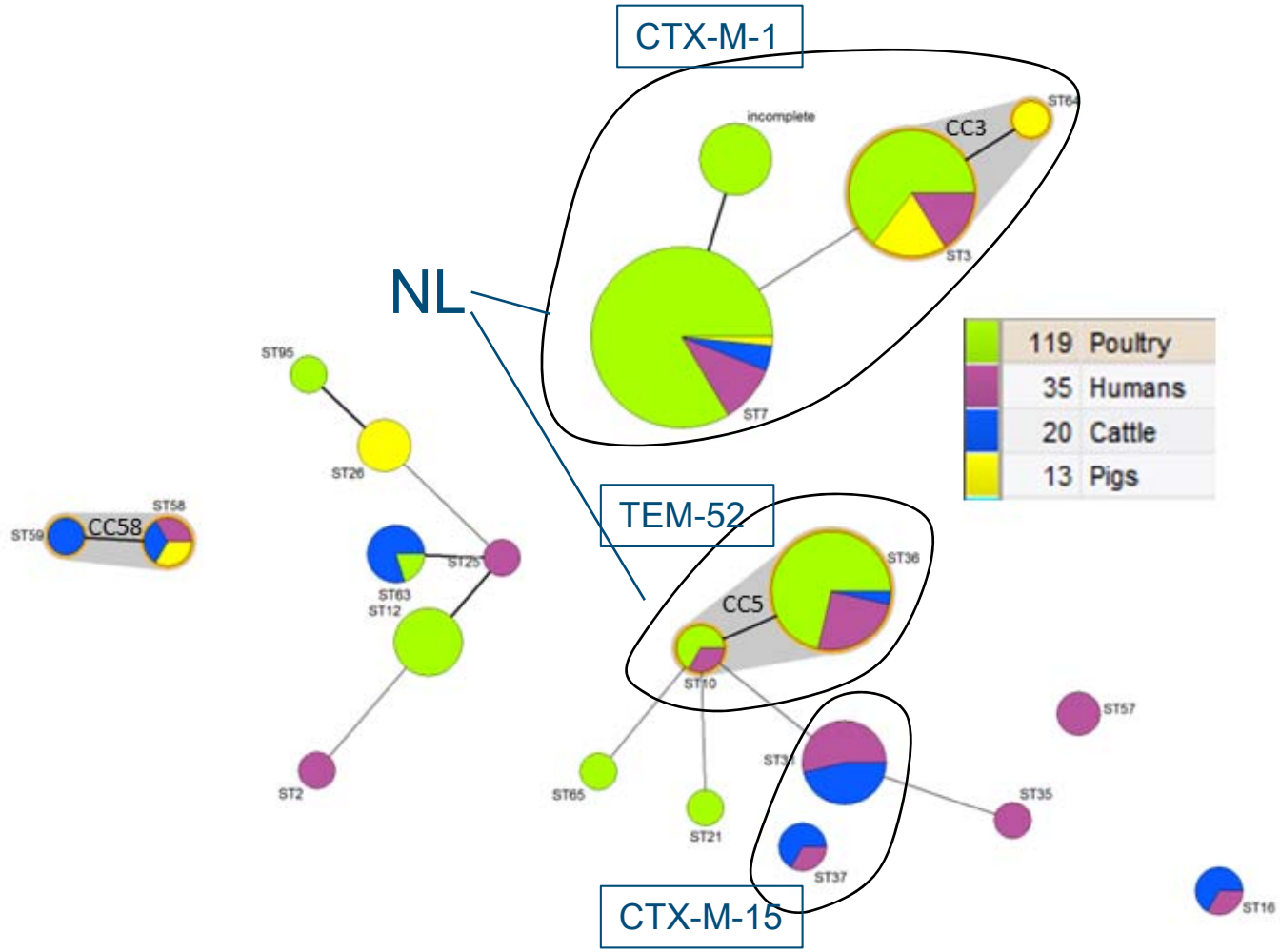


150	CTX-M-1
66	CMY-2
33	TEM-52
25	CTX-M-15
7	TEM-52c
4	CTX-M-3
3	CTX-M-15, TEM-1
3	SHV-12
3	CTX-M-1 group
2	TEM-20
2	CMY-23
1	TEM-52?
1	SHV-12, TEM-1
1	CTX-M-14
1	CTX.-M-1 group

MS vs gene of 189 incI1 plasmids



Animal versus human sources

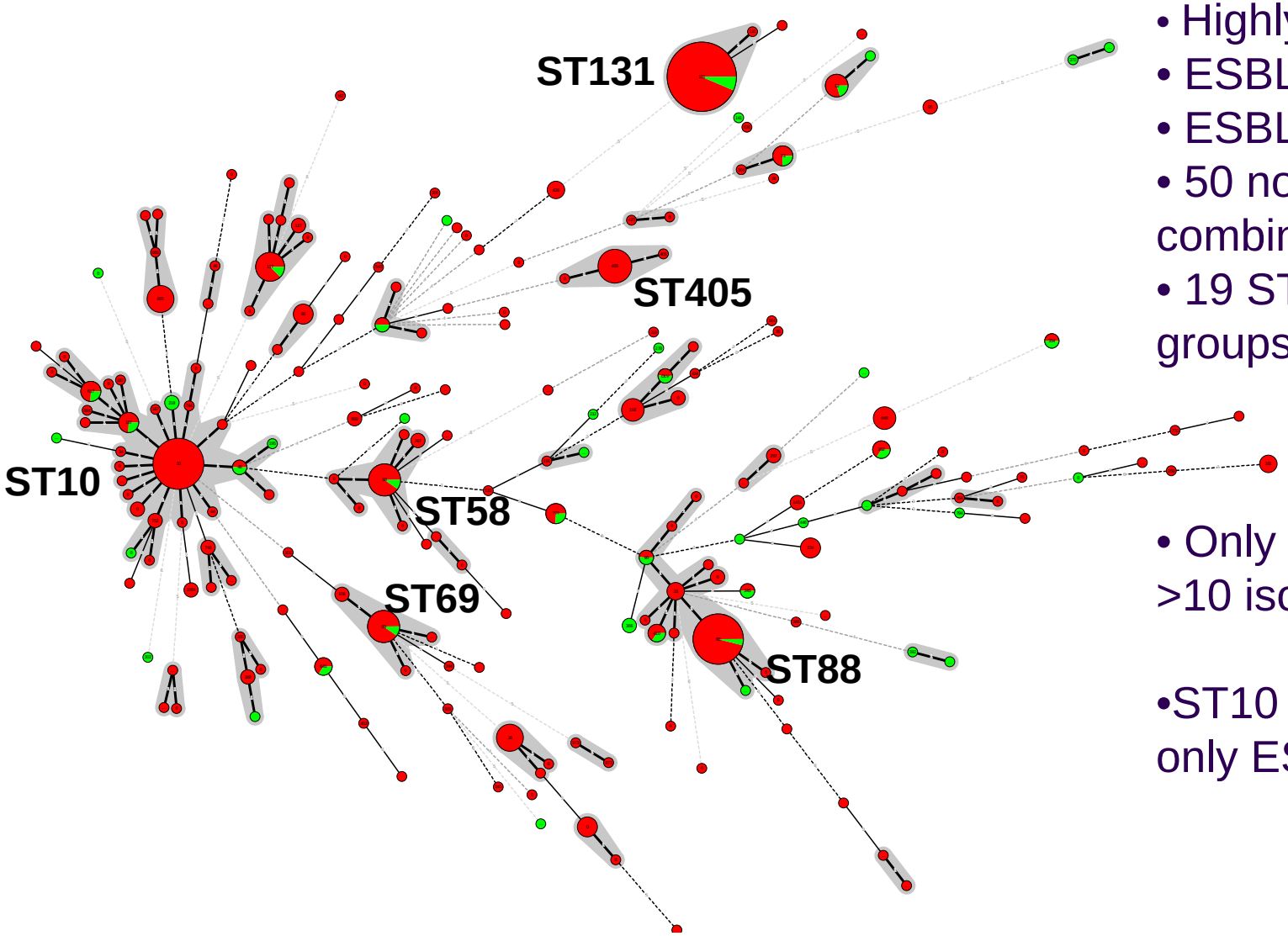


In conclusion

- pMLST analysis of a large collection incI1 plasmids from 2009 confirmed the genetic relatedness described previously between poultry and human incI1-plasmids
  - NL, (CTX-M-1, TEM-52)
  - Likely zoonotic nature

MS tree of STs:

ESBL +ve (red), ESBL -ve (green)



- Highly diverse
- ESBL +ve, 180 STs
- ESBL -ve, 46 STs
- 50 novel allele combinations
- 19 STs in both groups

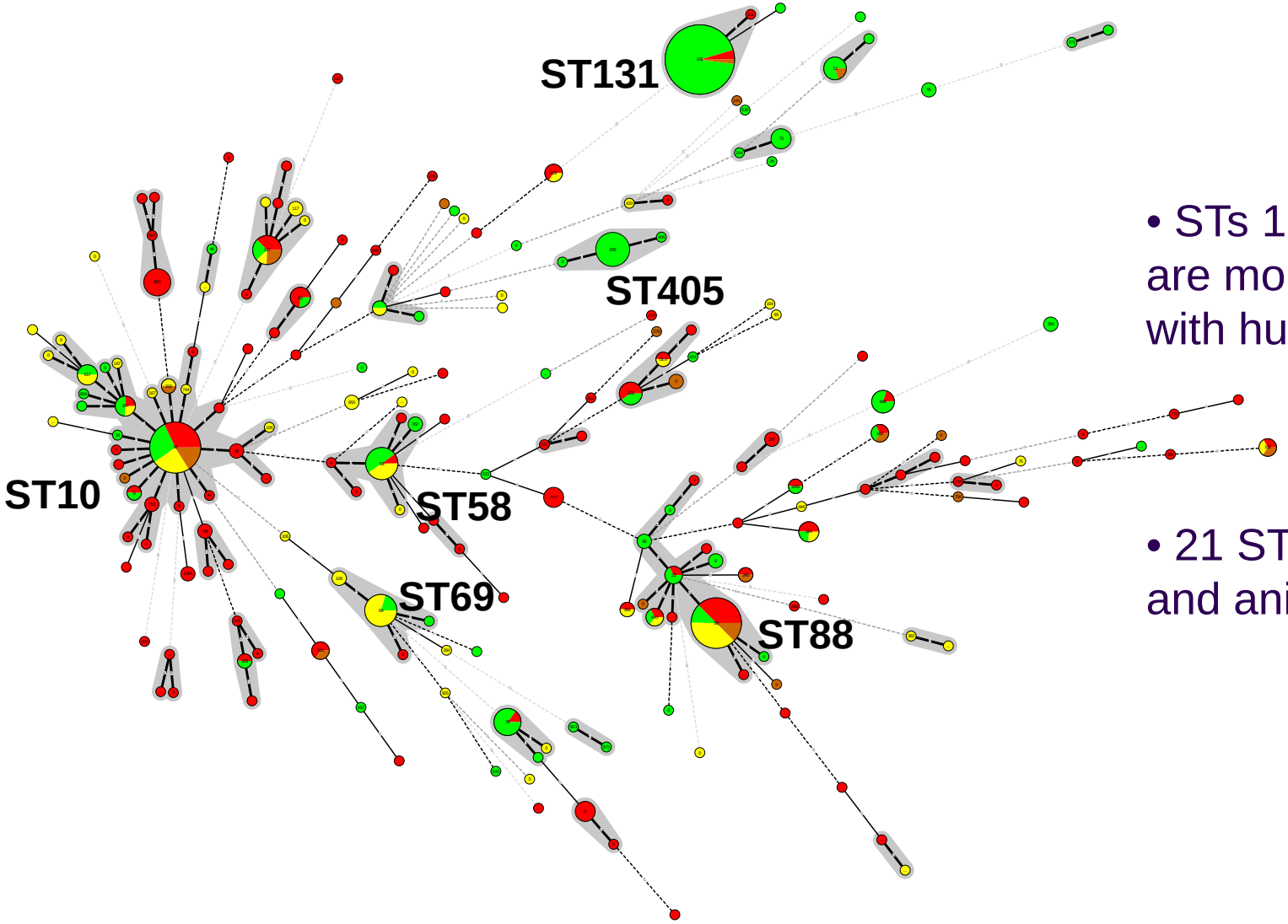
- Only six STs with >10 isolates

- ST10 and ST405 only ESBL producers

MS tree of STs *vs.* source species:

Poultry (red), human (green), cattle (yellow),

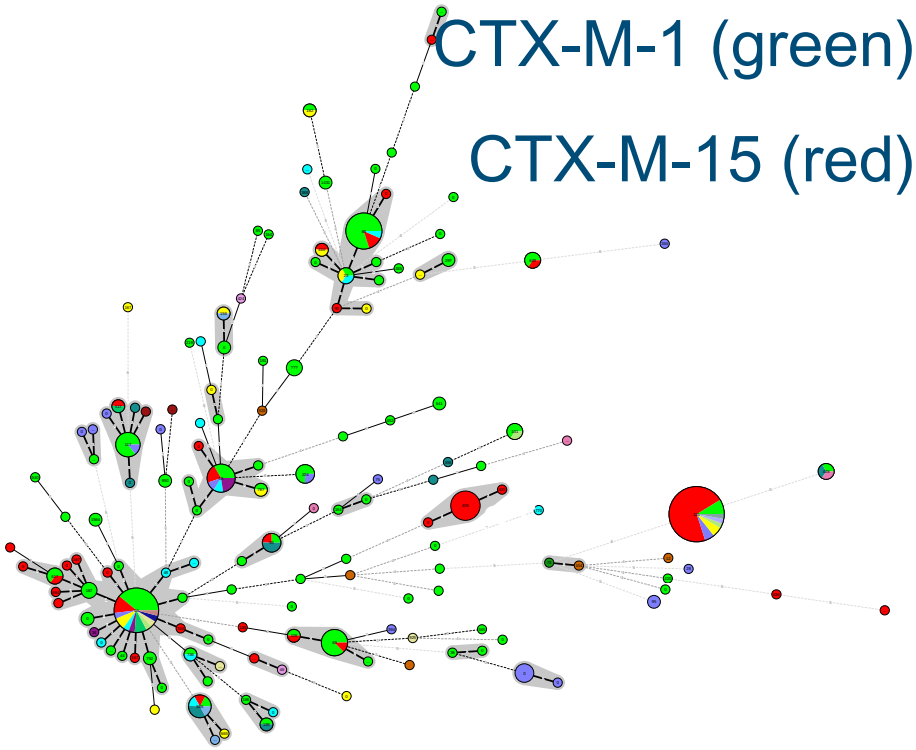
other (brown)

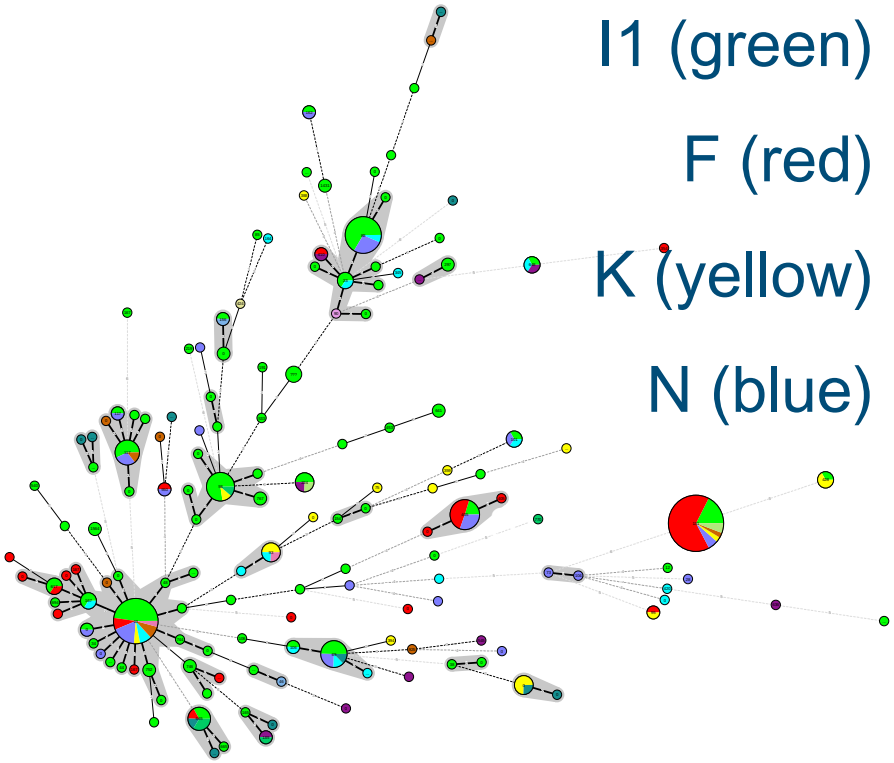


- STs 131 and 405 are more associated with humans
- 21 STs in humans and animals

MS trees of STs *vs.* ESBL and *rep* type

(294 transformants)





- Most major STs host multiple ESBLs (exception is ST405)
- IncI1 plasmids encoding CTX-M-1 are found throughout tree
- F plasmids encoding CTX-M-15 dominate in ST131
- ST405 hosts F, I1 and N plasmids, all encoding CTX-M-15

In conclusion

- Epidemiology of CTX-M-1 (TEM-52) predominantly determined by transmission of IncI1-plasmids in humans and animals

- Epidemiology of CTX-M-15 predominantly determined by clonal distribution of E. coli STs with F-plasmids in humans

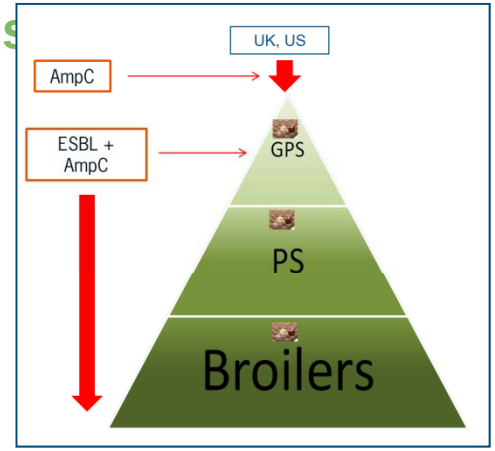
ESBLs in other animals in The Netherlands

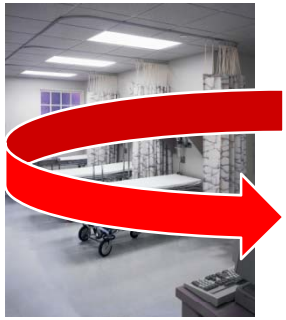
Animal species	ESBLs-Prevalence	ESBL subtypes detected
Companion anima		, 2, 9, 14, 15,
Slaughter pigs		EM- A-1
Veal calves		, 2, 14, 15, 32, CMY-2, OXA-1
Dairy cows	11% individual animals	CTX-M-1, 2
Turkeys	50% flocks	CTX-1,15, CMY-2

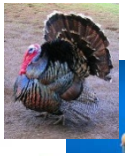
Wild bird	Species	ESBL
Wilde eend	*Anas platyrhynchos*	4
Rotsduif	*Columbia livia*	1
Kemphaan	*Philomachus pugnax*	1
Tureluur	*Tringa totanus*	5
Kokmeeuw	*Chroicocephalus ridibundus*	2
Zilvermeeuw	*Larus argentatus*	1
Zwarte zwaan	*Cygnus atratus*	1
Grote mantelmeeuw	*Larus marinus*	1
Jan van Gent	*Morus bassanus*	1
Total		17 (22%)

Is poultry the source or part of the problem??

Transmission to humans







BLs a zoonoses?

es, but attribution from animals depends on ountry and ESBL-gene

urrent concerns in Dutch health care

- ESBL-producing E. coli (CTX-M-15 dominant?)
- OXA-48 in Klebsiella, and E. coli
- VIM-2 in P. aeruginosa
- NDM-1 in Enterobacteriaceae
- KPC in Klebsiella

knowledgements

r. A. Carattoli

- IncI1-pMLST curator

PA-Colindale

- John Threlfall, John Wain, Neil Woodford, Michaela Williams

HVLA-Weybridge

- Nick Coldham, Guanghui Wu, Martin Woodward

- BfR-Berlin
  - Beatriz Guerra, Irene Rodrigues-Fernandez, Janine Beutlich, Reiner Helmuth
- FLI-Mariensee
  - Anne-Katrin Schinck, Kristina Kadlec, Stefan Schwarz
- UMCU-Utrecht
  - Maurine Leverstein-van Hall and colleagues