



Rijksinstituut voor Volksgezondheid
en Milieu
*Ministerie van Volksgezondheid,
Welzijn en Sport*



One Health plasmid analyses:
similarities of ESBL plasmids between farm
animals and the Dutch population

Michael Visser

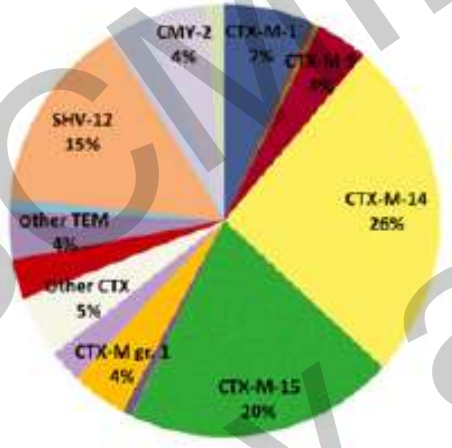


ESBL and AmpC prevalence

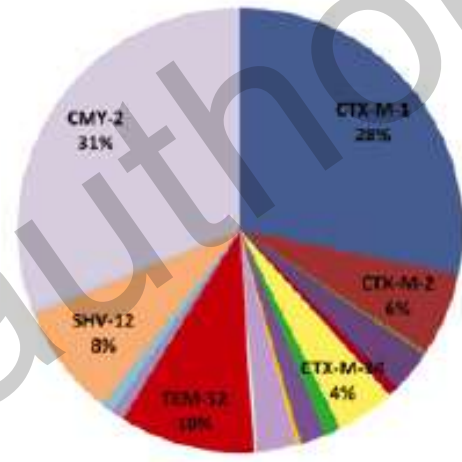
~5 % of the general Dutch population has an Extended spectrum beta-lactamase- or AmpC beta-lactamase- producing *E. coli* (ESBL-E)

Europe

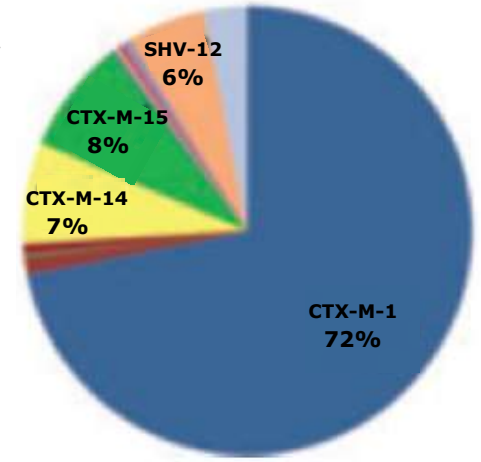
humans



poultry

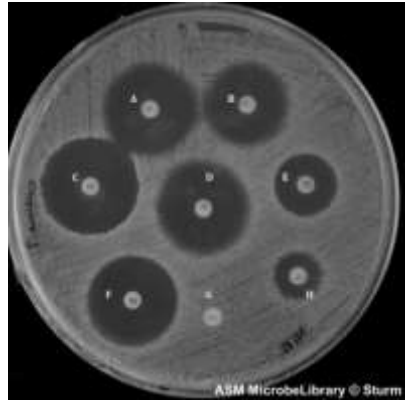
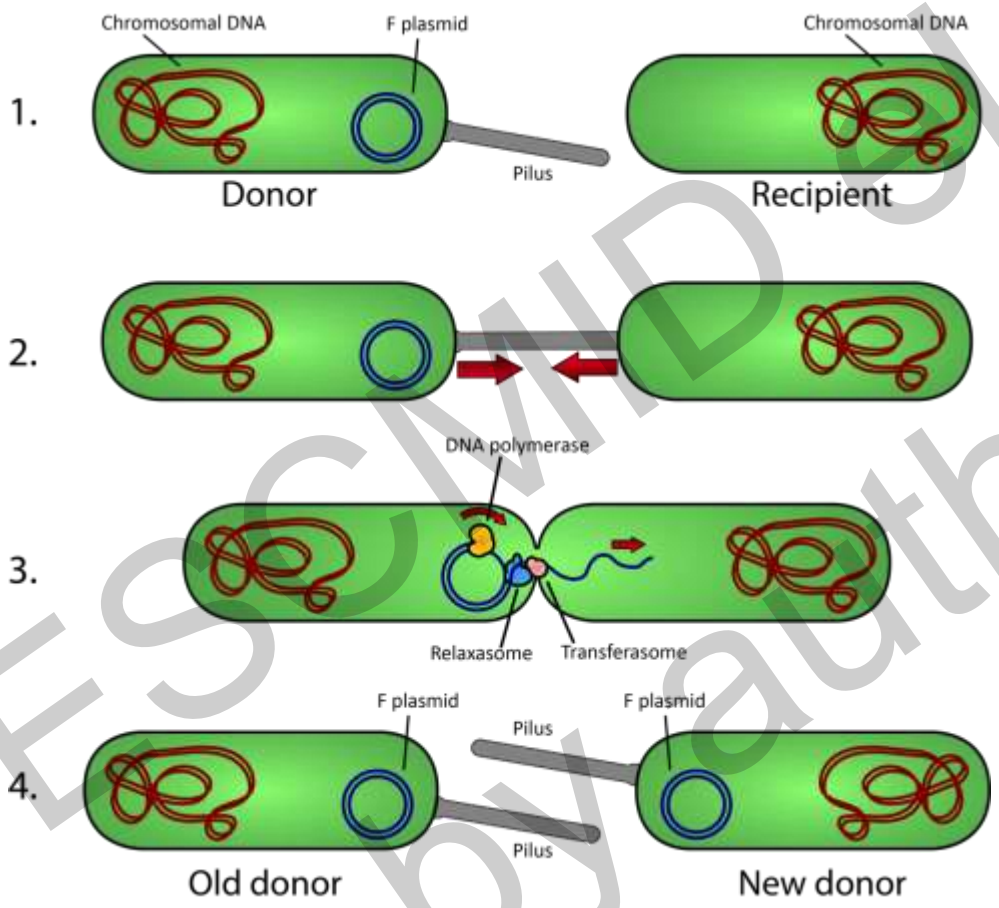


Cattle/pigs



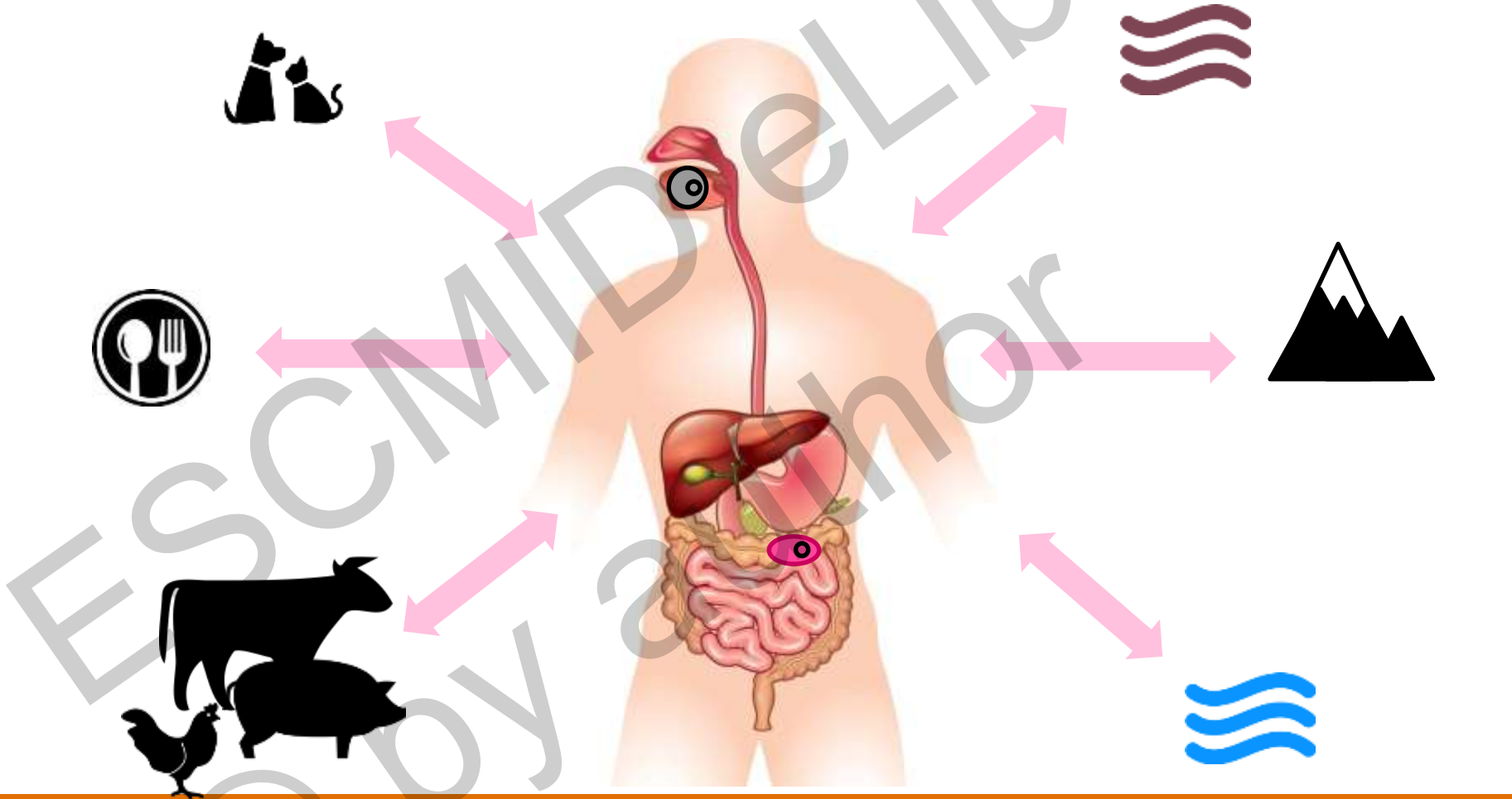


Conjugation





Clonal and horizontal transmission





Aim

- To get a better understanding of the contribution of livestock to ESBL-E carriership in humans
- Our hypothesis is that plasmids play a more important role than clonal transmission



Strain selection for WGS

Source	CTX-M-1	CMY-2	SHV-12	TEM-52	Total
Broilers	39	34	8	1	82
Broiler farmers	8	14	3	1	26
Goats	2	0	1	0	3
Goat farmers	2	0	0	0	2
Laying hens	56	30	0	0	86
Laying hen Farmers	0	0	0	0	0
Pigs	56	1	0	2	59
Pig Farmers	6	0	0	0	6
Dutch population	27	15	8	2	52
Total	196	94	20	6	316



Methods

- Illumina sequencing technology

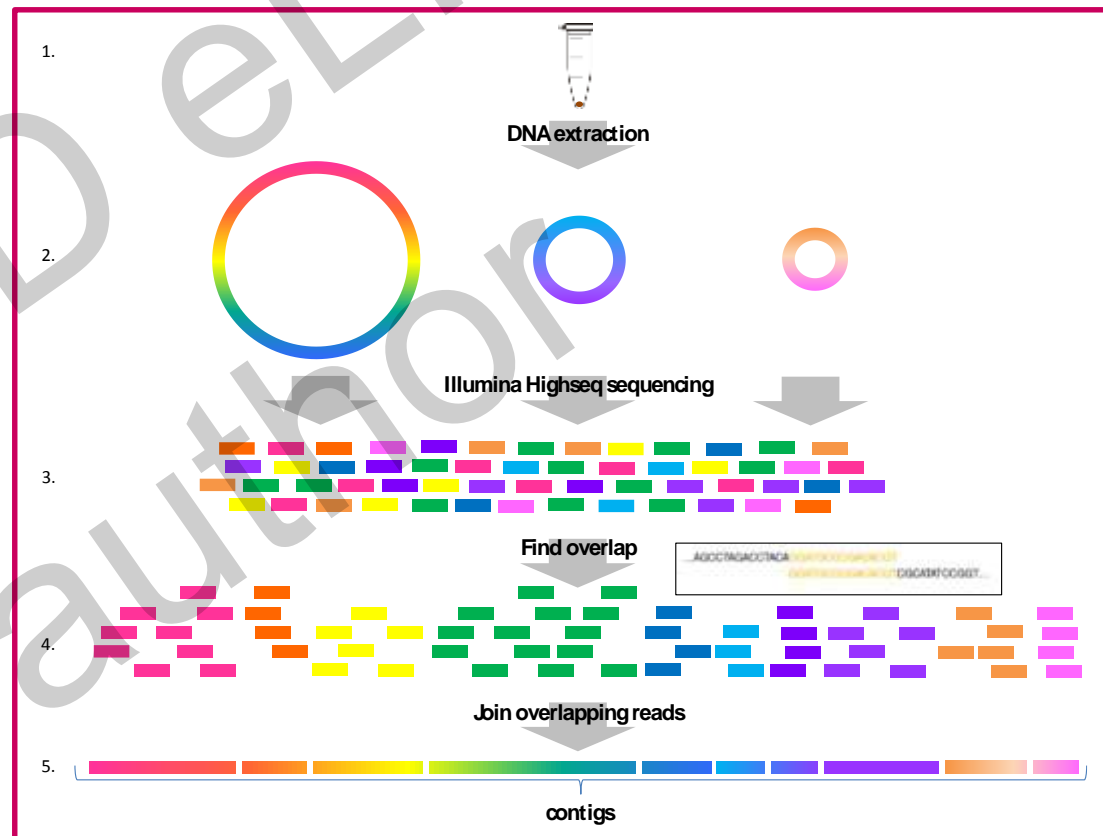
- HiSeq2500
- paired-end
- ~125 bp

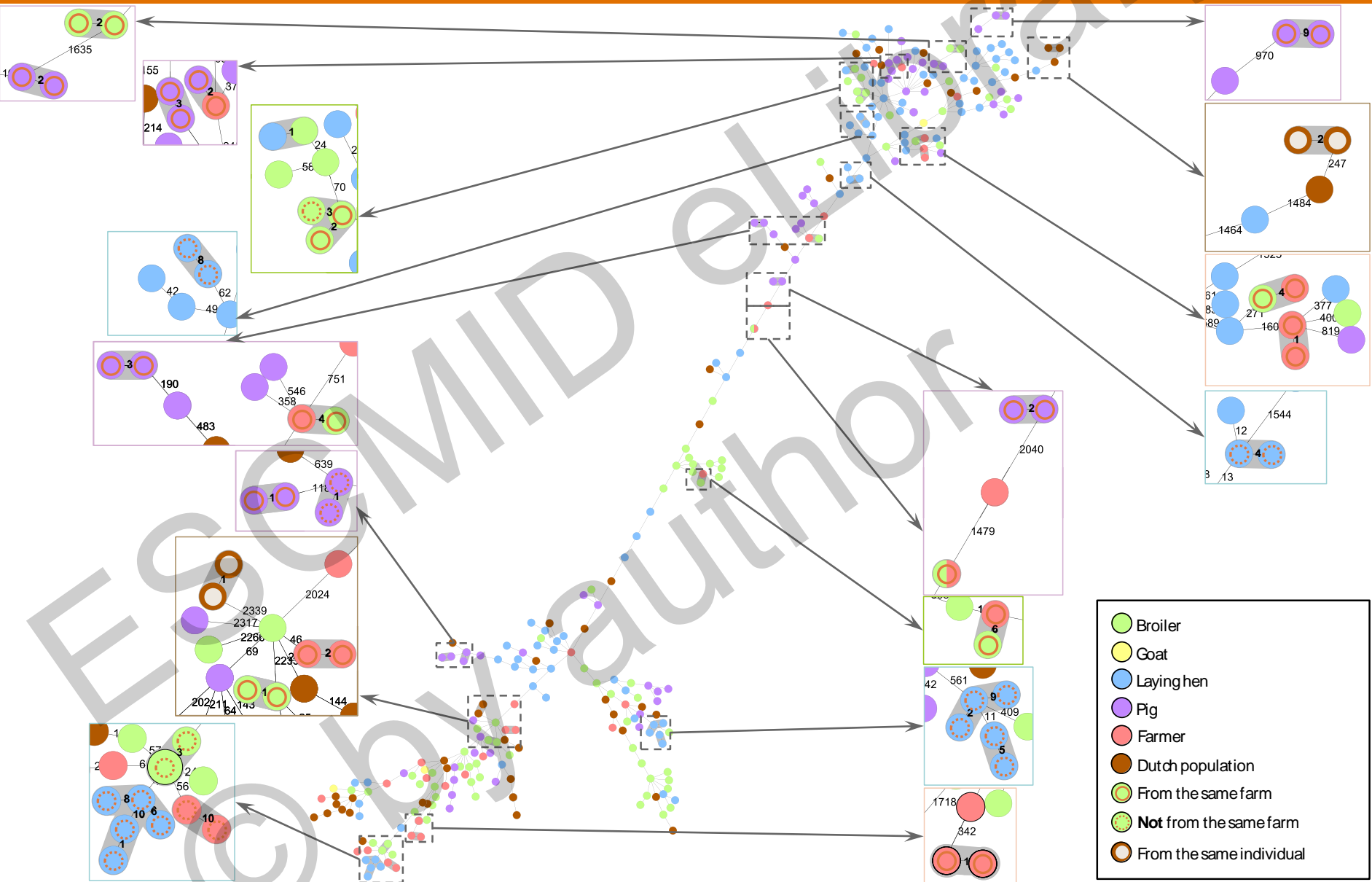
- Assembly

- Spades

- Analyzing chromosomes

- cgMLST
- Scheme of ~2500 genes







Summary cgMLST

Clonal link between:

- Farm animals on the same farm
 - Farm animals and farmers on the same farm
 - Similar farm animal species but on different farms
 - Different farm animal species
-
- *No clonal link between ESBL-E from livestock and the general Dutch population was observed in this study*



Fishing for plasmids



BLAST

- Database
- 210 *E. coli* chromosomes
 - 2510 plasmids

Query	1	AATTACAGGCAAACATCGGCAGATTCTCAAGGGATATTTAATATTGTTGGCTGGAATAG	60
Sbjct	141	AATTACAGGCAAACATCGGCAGATTCTCAAGGGATATTTAATATTGTTGGCTGGAATAG	200
Query	61	TAGTTACACGGGGGAACCTATCCCGGTTGCTCAGATGCGAGAATGGCTAGATGATAAAGT	120
Sbjct	201	TAGTTACACGGGGGAACCTATCCCGGTTGCTCAGATGCGAGAATGGCTAGATGATAAAGT	260
Query	121	TAAGGTTATTCTCGCTCAAAAACCGAAAAAGTTCTGGAAATAGGTTGTGGAACCGGGTT	180
Sbjct	261	TAAGGTTATTCTCGCTCAAAAACCGAAAAAGTTCTGGAAATAGGTTGTGGAACCGGGTT	320
Query	181	AATATTATTCCAAGTTGCTCCCCATTGCCAGTGTTATTGGGGAACCGATATTTTCATCAGT	240
Sbjct	321	AATATTATTCCAAGTTGCTCCCCATTGCCAGTGTTATTGGGGAACCGATATTTTCATCAGT	380
Query	241	AGCCTTAGACCATATTCAGCGAATTAATCAAGAAGGGCCTCAGCTAGAGCAAGTCAGGCT	300
Sbjct	381	AGCCTTAGACCATATTCAGCGAATTAATCAAGAAGGGCCTCAGCTAGAGCAAGTCAGGCT	440
Query	301	ATTGCATAGCACAGCCGATAATTTTGAGGGTTTGGAGTCAGAAGGATTCGATACAATTAT	360
Sbjct	441	ATTGCATAGCACAGCCGATAATTTTGAGGGTTTGGAGTCAGAAGGATTCGATACAATTAT	500

chromosome

plasmid A

plasmid B

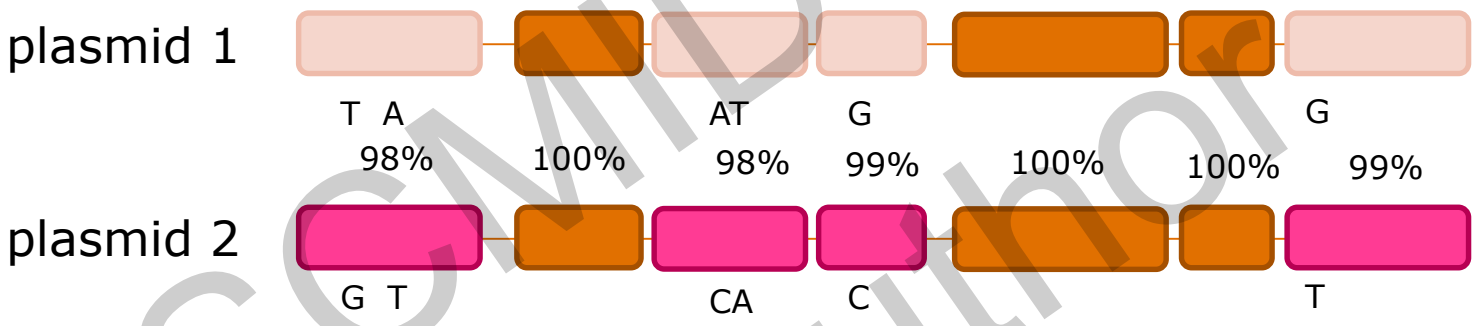




Plasmid comparisons

genes vs genes

- Basic Local Alignment Search Tool (BLAST)





CTX-M-1 carrying plasmids

Plasmid type	Broiler	Goat	Laying hen	Pig	Farmers	Dutch population	total
Incl1-ST-3	21		43	19	6	4	93
Incl1-ST-7	11			15	3	3	32
Incl1-Unknown	3		8	2	2	1	16
Incl1-ST-58				3		7	10
InclN-ST-1					1	5	6
InclFI				2		1	3
Incl1-ST-157	1		1		1		3
InclFIA		2					2
Incl1-ST-35	1					1	2
Incl1-ST-49				2			2
Incl1-ST-63				2			2
Incl1-ST-295	1		1				2
InclFIB			1				1
InclFIC				1			1
Incl1-ST-42	1						1
InclX1						1	1



IncI1-ST-3

< 4 genes difference





IncI1-ST-7

< 4 genes difference





IncI1-ST-58

< 4 genes difference



- Broiler
- Goat
- Laying hen
- Pig
- Farmer
- Dutch population
- From the same farm
- **Not** from the same farm
- From the same individual



CMY-2 carrying plasmids

Plasmid type	Broiler	Laying hen	Farmers	Dutch population	total
Inck	22	9	6	7	44
Incl1-ST-12	10	9	8	2	29
Incl1-Unknown		5			5
IncAG-ST-3	2				2
IncFIB	2				2



Inck

< 4 genes difference



ESCMID eLibrary
© by author



Summary plasmid analyses

Similar plasmids found in:

- Farm animals on the same farm
- Farm animals and farmers on the same farm
- Similar farm animal species but on different farms
- Different farm animal species

- *Livestock and the general Dutch population*



Conclusions

- Clonal transmission of ESBL-E is indicated between different animal species, between farms, and between animals and humans with professional contact
- Plasmid transmission is indicated between ESBL-E of different animal species, farms, and animals and humans with and without professional contact
- The transmission routes will have to be investigated.
- When investigating possible transmission events of ESBL-E it is important to study horizontal plasmid transmission in addition to *E. coli* clonal transmission.



Thanks to

- Moniek van Selst
- Angela H. A. M. van Hoek
- Cindy Dierikx
- Engeline van Duijkeren





Plasmid Comparison

- Gene prediction – Prodigal
- Comparing genes of plasmids (similar plasmid type) – blastn

- Identity (sequence similarity)
- Genes on contig edges were left out of the comparison

Query	1	AATTACAGGCAACATCGGCAGATTCTCAAGGGATATTTAATATTGTTGGCTGGAATAG	60
Sbjct	141		200
Query	61	TAGTTACACGGGGAAACCTATCCCGGTTGCTCAGATGCGAGAATGGCTAGATGATAAAGT	120
Sbjct	201		260
Query	121	TAAGTTATTCTCGCTCAAAAACCGAAAAAGTTCTGGAATAGGTTGTGGAACCGGGTT	180
Sbjct	261		320

