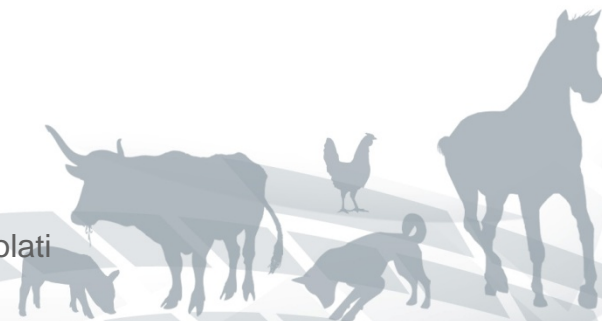




ISTITUTO G. CAPORALE
TERAMO

Baseline study 2008: caratterizzazione dei ceppi isolati

Baseline study 2008: caratterizzazione dei ceppi isolati
Alessandra Alessiani



Campioni pervenuti al Laboratorio Nazionale di Riferimento per *Campylobacter* 2008-2009.

- 693 dei 741 campioni pervenuti al LNRC appartenevano al baseline study del 2008.

PROVENIENZA	CARCASSE POLLO	FECI POLLO	LATTE BOVINO	UMANO	NON NOTO	TOTALE
IZS ABRUZZO & MOLISE	44	5	1	2	10	62
IZS SICILIA	10	10				20
IZS VENEZIE	205	132			9	346
IZS LOMBARDIA ED EMILIA ROMAGNA	46	101			2	149
IZS UMBRIA E MARCHE	45	31	3	11	9	99
IZS MEZZOGIORNO	14	8				22
IZS PIEMONTE, LIGURIA E VALLE D'AOSTA	11	31			1	43
TOTALE	375	318	4	13	31	741



Identificazione e Caratterizzazione dei ceppi.

- Polymerase Chain Reaction (Wang et al. 2002).
- Pulsed Field Gel Electrophoresis (Pulsenet 2008).
- s-AFLP (Champion et al. 2002).
- Emoagglutinazione passiva, PHA (Patton et al. 1985).
- Antibiogramma e Sequenziamento del QRDR (Zirnstein 1999) .



Polymerase Chain Reaction

Wang et al. JCM 2002, Vol. 40 No. 12.

- *C. jejuni*: hip0
- *C. coli*, *C. lari*, *C. upsaliensis*: glyA
- *C. fetus*: sapB2

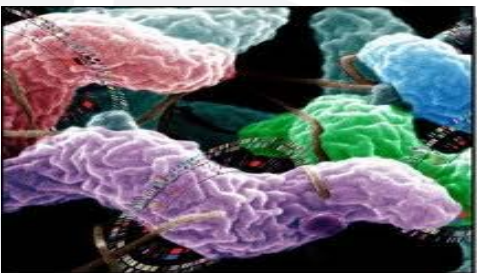


pHA

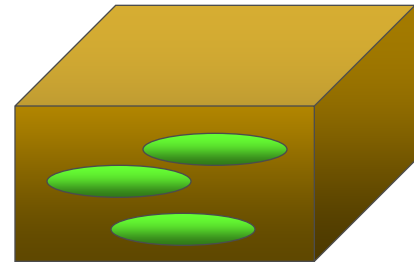
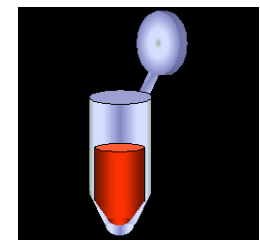
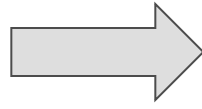
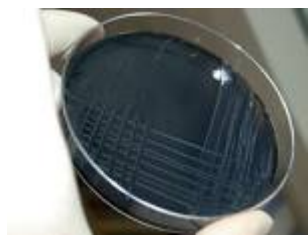


- Sierotipizzazione secondo il metodo di Penner.

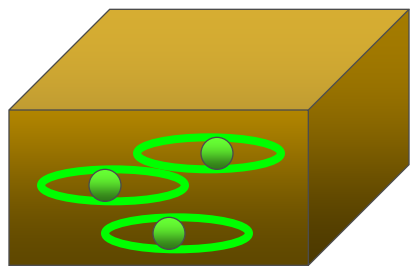
Campylobacter jejuni



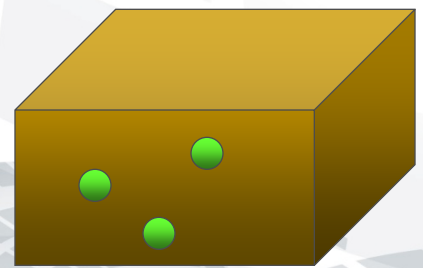
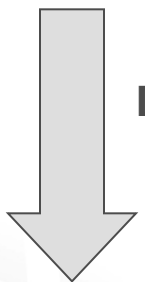
Pulsed Field Gel Electrophoresis



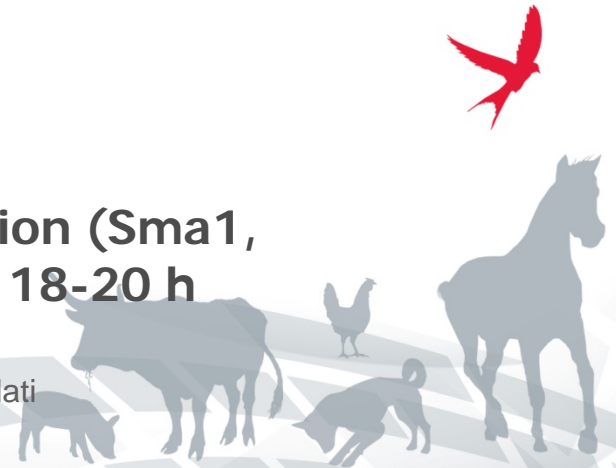
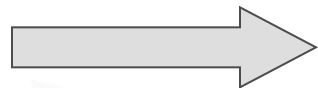
Proteinase K



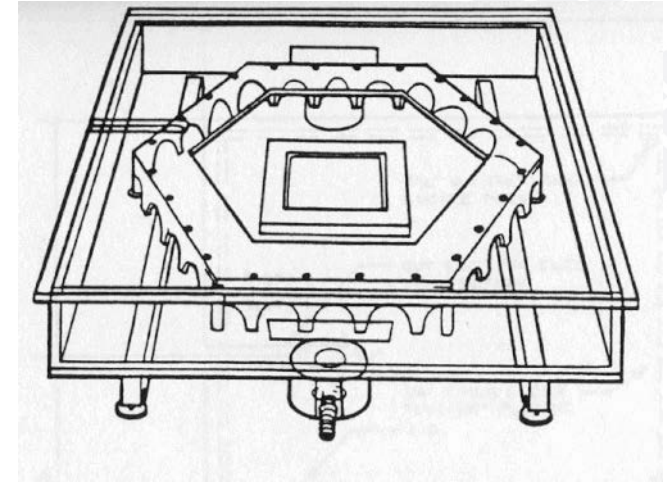
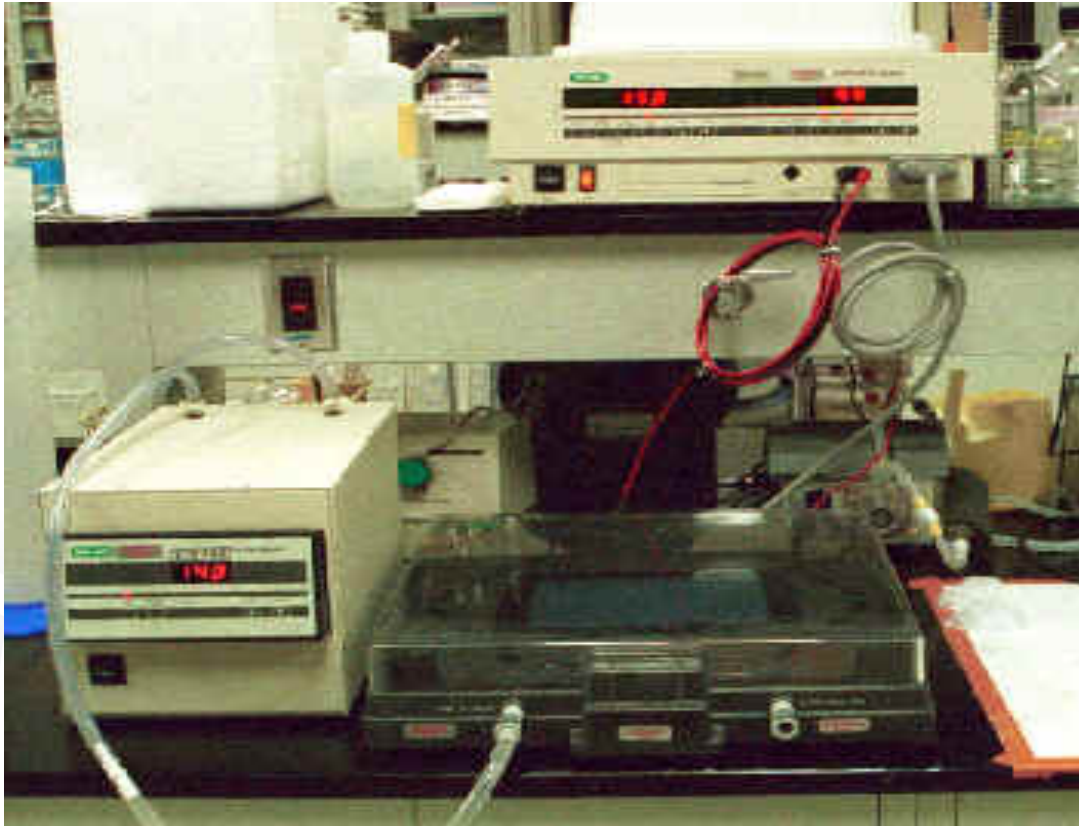
DNA extraction and washing
to eliminate residual



Enzyme restriction (Sma1,
Kpn1, Xba1) 18-20 h



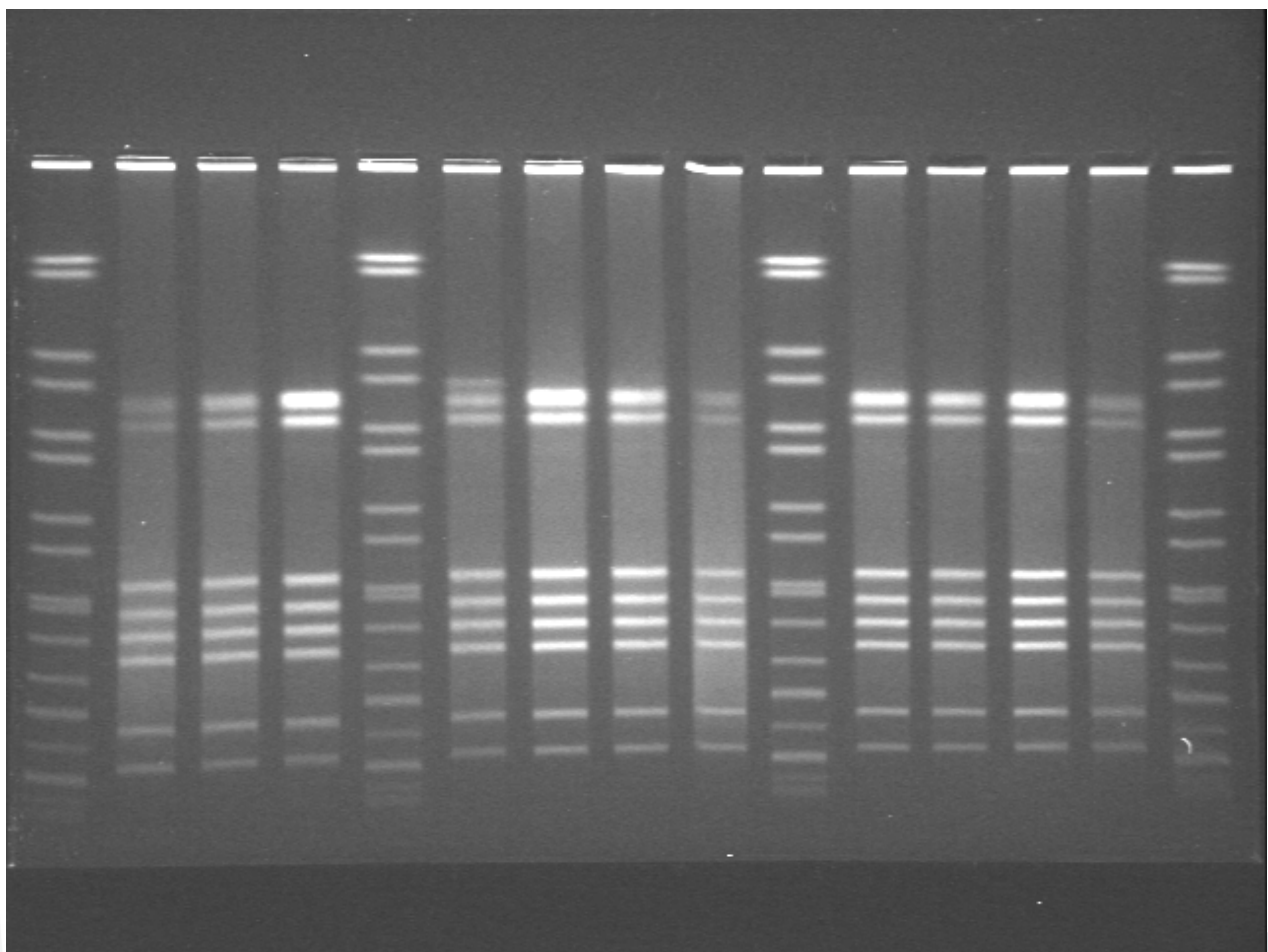
Pulsed Field Gel Electrophoresis



Baseline study 2008: caratterizzazione dei ceppi isolati
Alessandra Alessiani



Pulsed Field Gel Electrophoresis

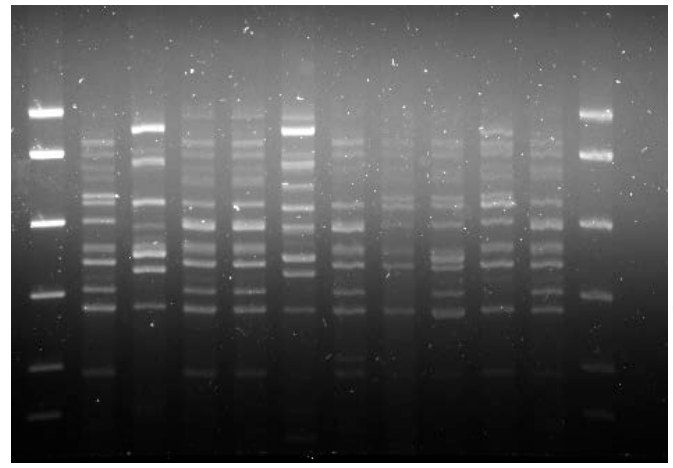
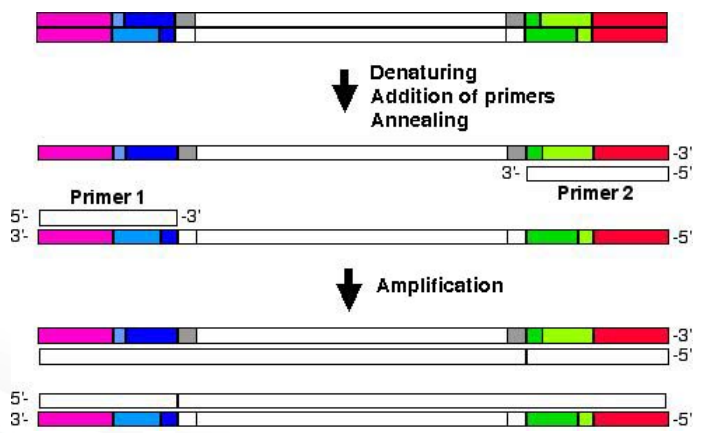


Baseline study 2008: caratterizzazione dei ceppi isolati
Alessandra Alessiani

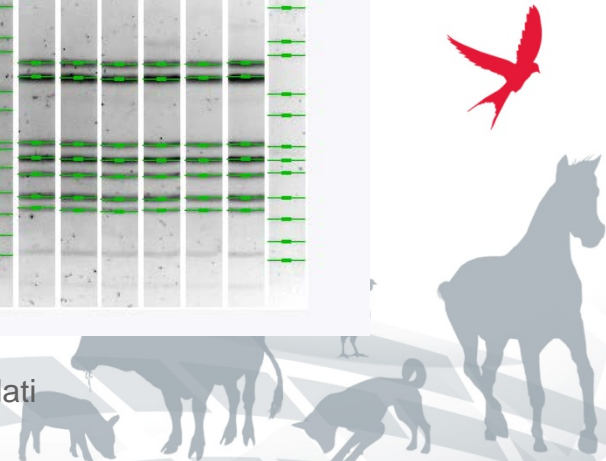
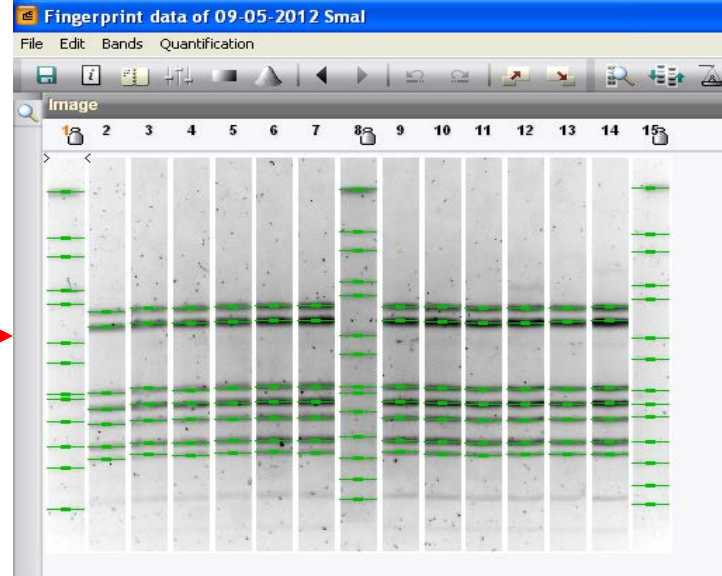
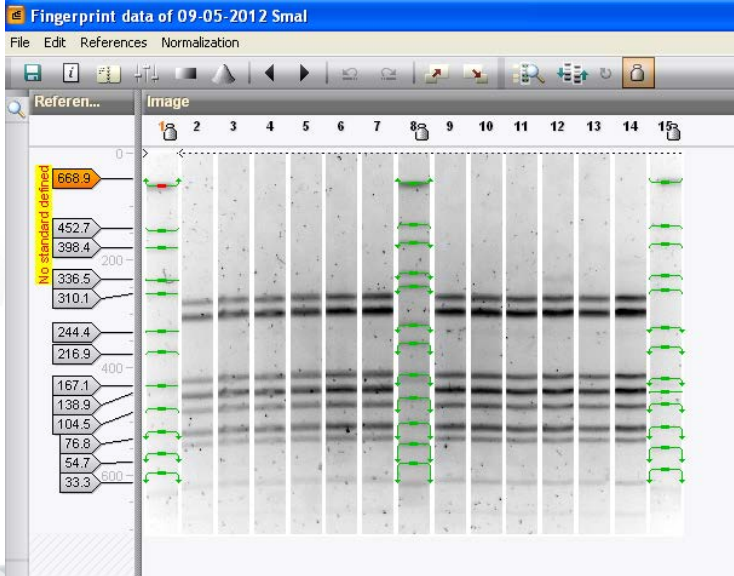
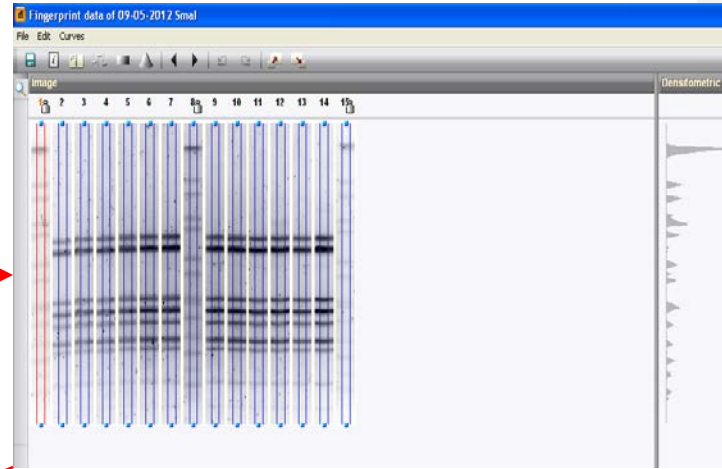
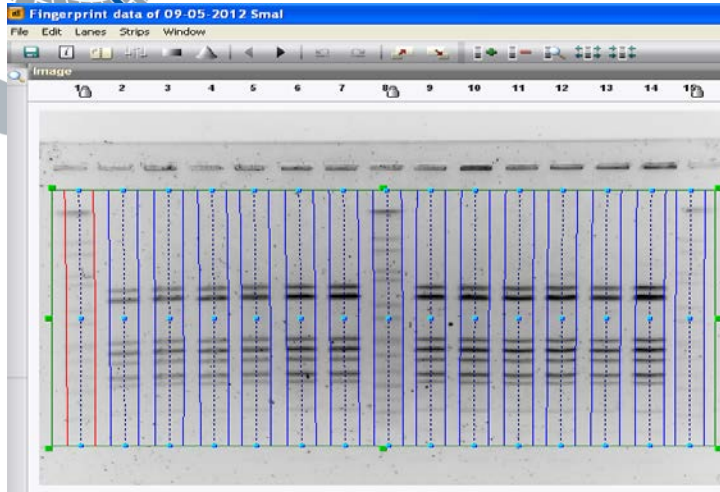


Single Amplified Fragment Length Polymorphism

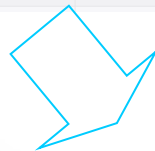
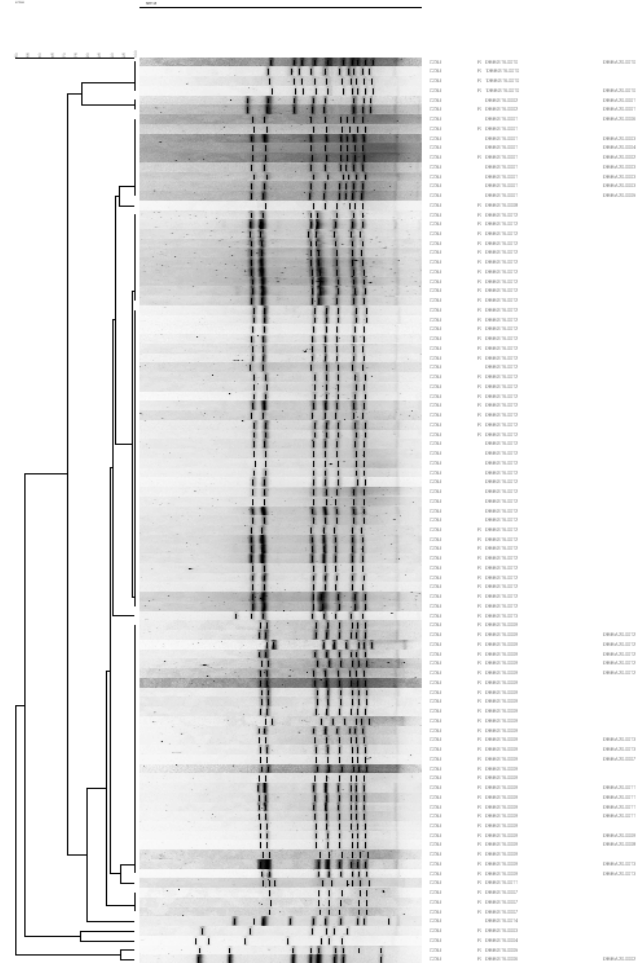
- s-AFLP



BIONUMERICS 6.5.



Key	HIRG	PCR Multiplex Conferma
1000.0	150444 PE	Negativo
1001.0	150446 PE	Negativo
1002.0	150447 PE	Negativo
1003.0	150450 PE	COLI
1004.0	7220 CB	COLI
1005.0	7220 CB	COLI
1006.0	7220 CB	COLI
1007.0	7224 CB	COLI
1008.0	7226 CB	COLI
1009.0	7227 CB	COLI
101.0	1272641	Negativo
1010.0	72210 CB	COLI
1011.0	72216 CB	COLI
1012.0	72217 CB	COLI
1013.0	72218 CB	COLI
1014.0	72219 CB	COLI
1015.0	72220 CB	COLI
1016.0	72223 CB	COLI
1017.0	72224 CB	COLI
1018.0	72225 CB	COLI
1019.0	72226 CB	COLI
102.0	1272642	Negativo
1020.0	72250 CB	COLI
1021.0	72251 CB	COLI
1022.0	72252 CB	COLI
1023.0	72254 CB	COLI
1026.0	72257 CB	COLI
1027.0	72259 CB	COLI
1028.0	72240 CB	COLI
103.0	1272643	COLI
1030.0	72246 CB	COLI
1031.0	72247 CB	COLI
1032.0	72250 CB	COLI
1033.0	7228 CB	Negativo
1034.0	72295 CB	Negativo
1035.0	72296 CB	Negativo
1036.0	72244 CB	Negativo
1037.0	74771	JEJUNI



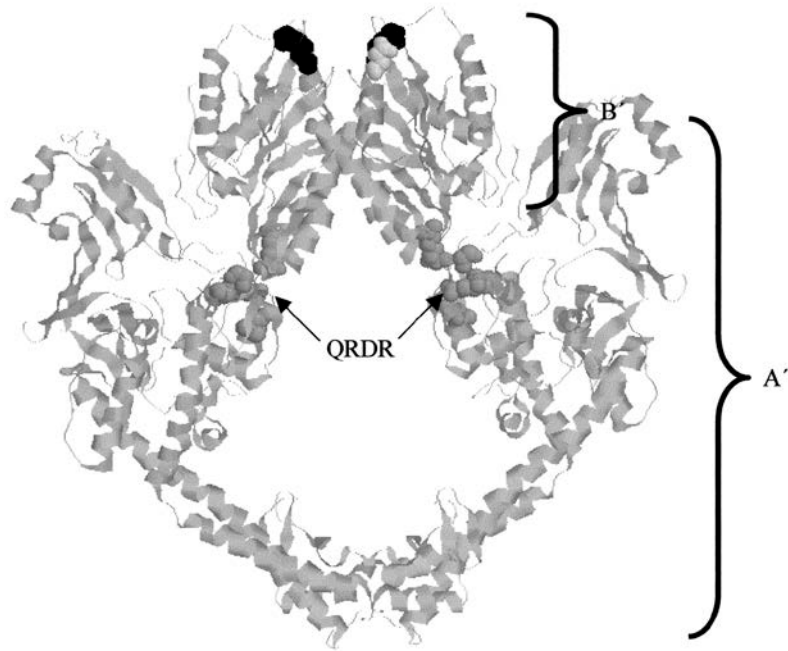
Baseline study 2008: caratterizzazione dei ceppi isolati
Alessandra Alessiani



Quinolone Resistance Determining Region

QRDR

B

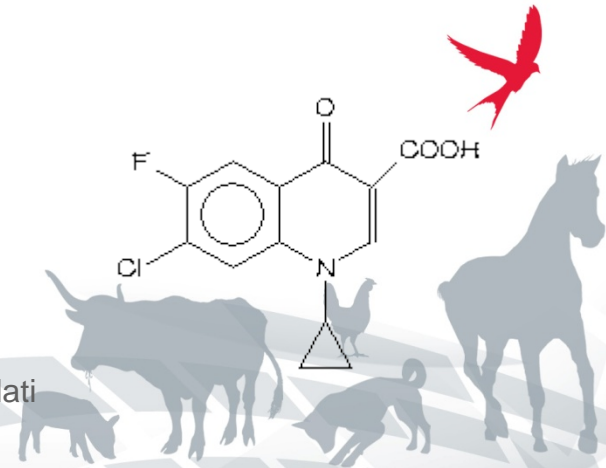
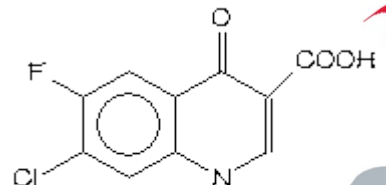


▶ T86 → I



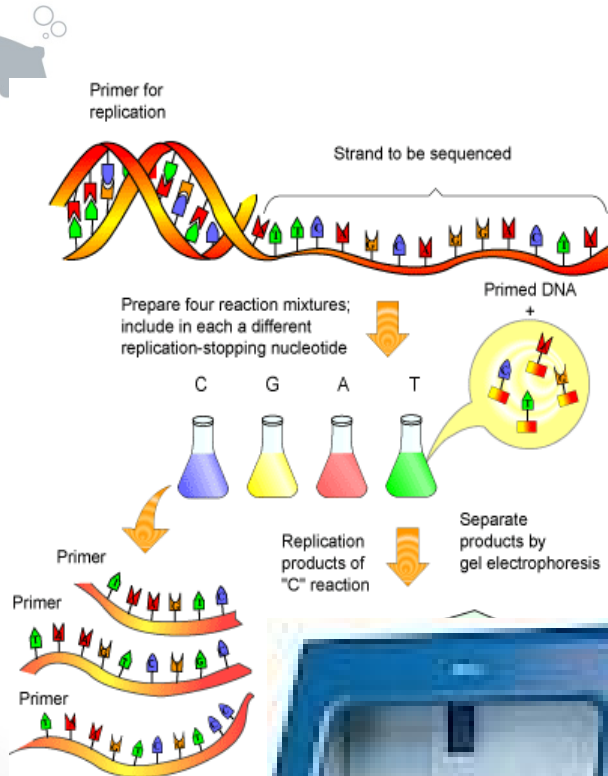
INDUZIONE DI RESISTENZA

- Da 0,02 $\mu\text{g/ml}$ a 64 $\mu\text{g/ml}$
- Antibiogramma
- Sequenziamento



SEQUENZIAMENTO

1. P.C.R.
2. Reazione di sequenza.
3. Sequenziamento.
4. Allineamento delle sequenze.



caratterizzazione dei ceppi isolati
Assandra Alessiani



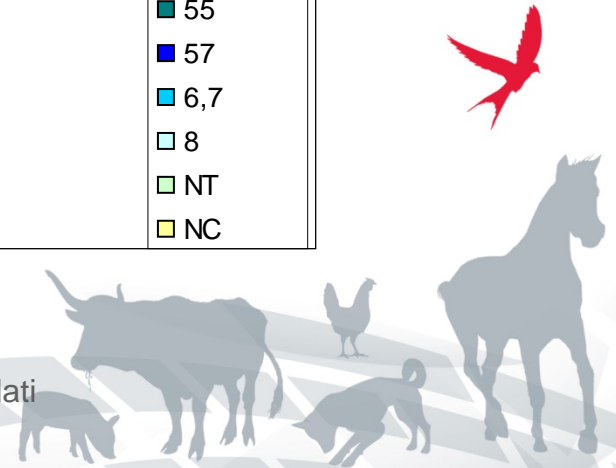
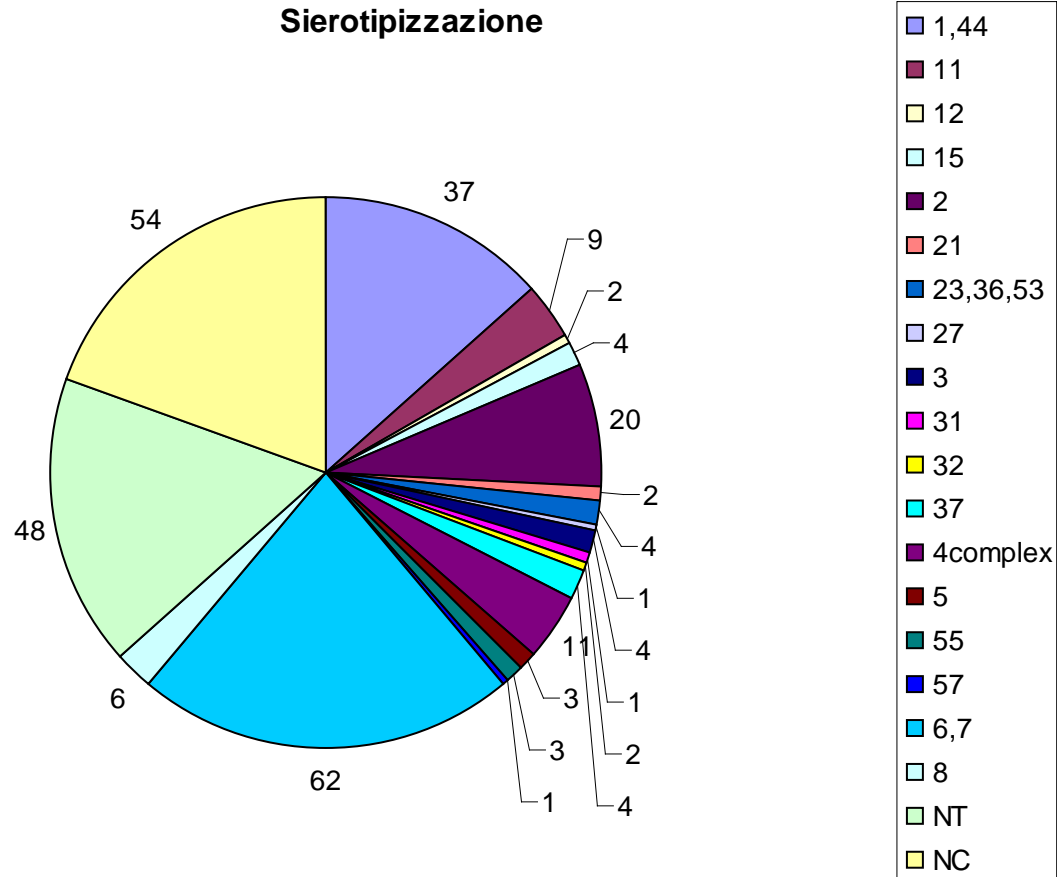
Risultati PCR

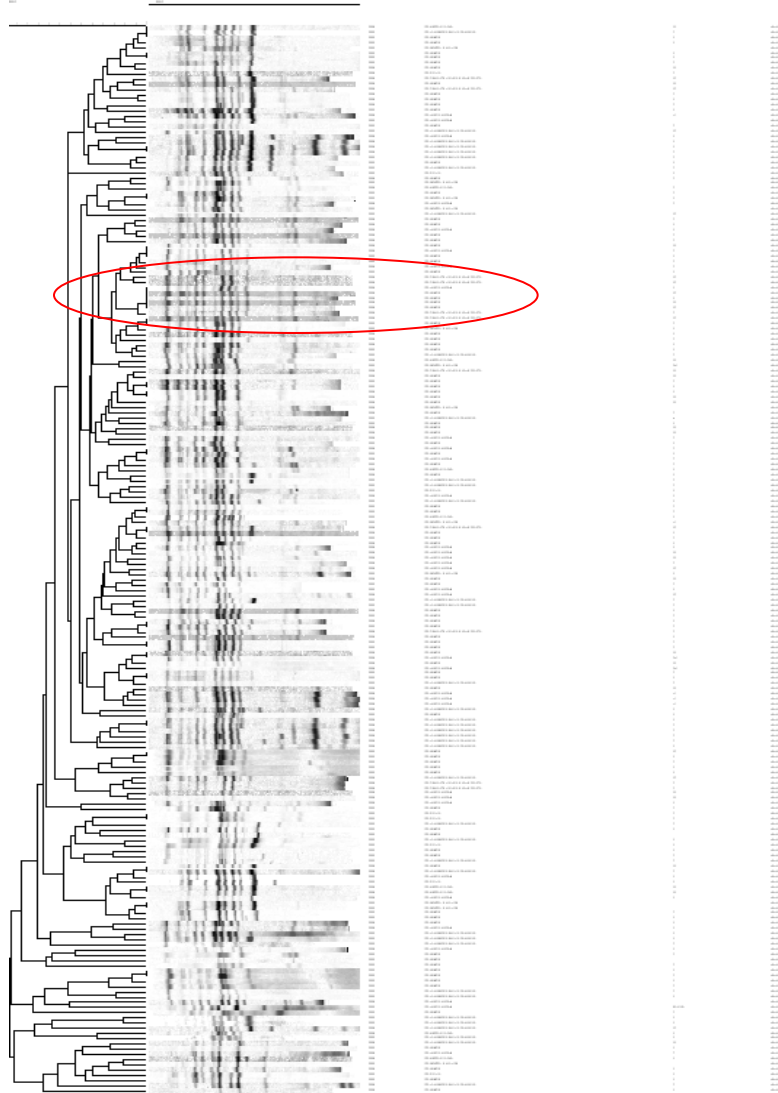
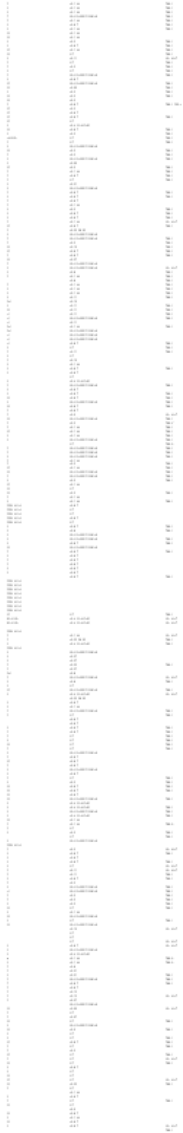
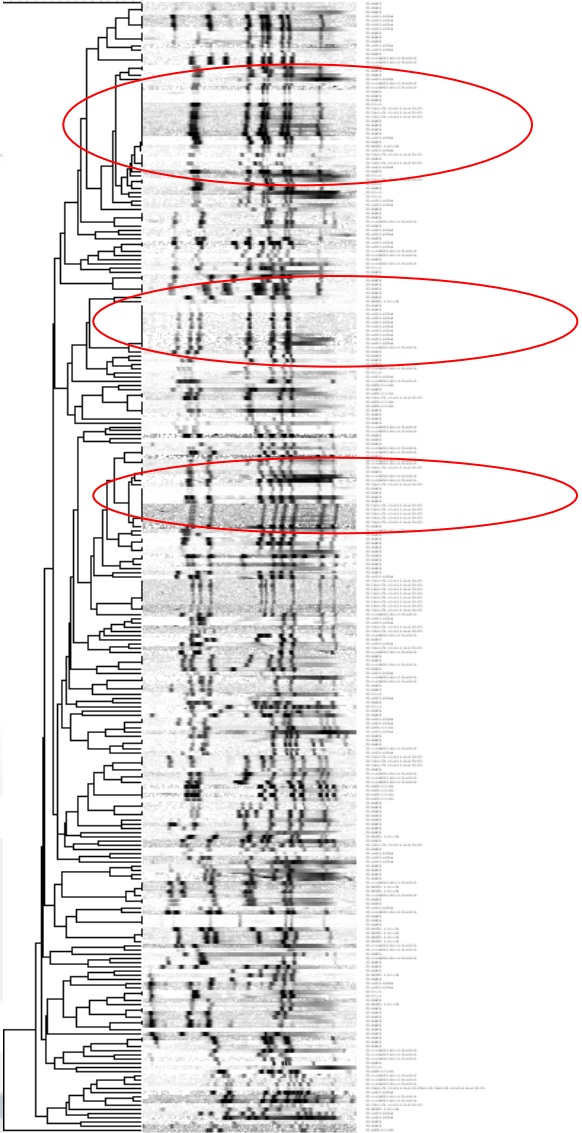
Campylobacter jejuni	195
Campylobacter coli	232
Campylobacter lari	3



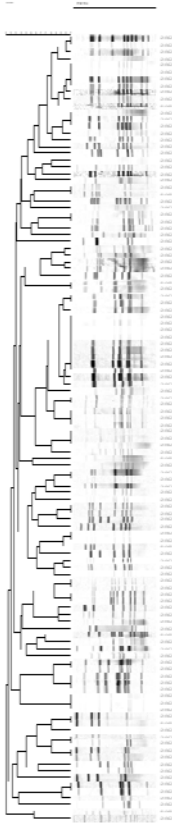
pHA

Sierotipizzazione

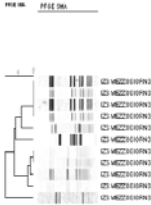




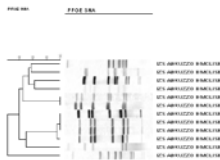
IZS VENEZIE



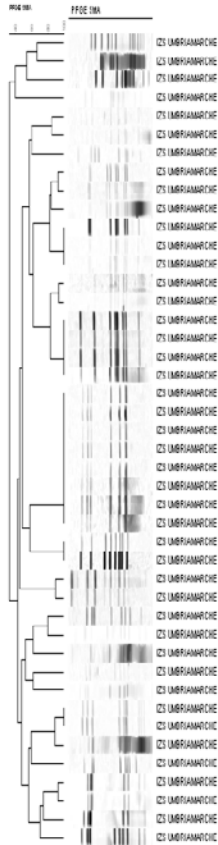
IZS MEZZOGIORNO



IZS ABRUZZO MOLISE



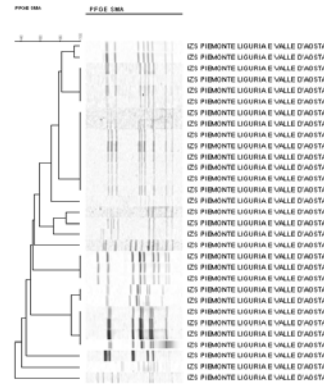
IZS UMBRIA MARCHE



IZS SICILIA

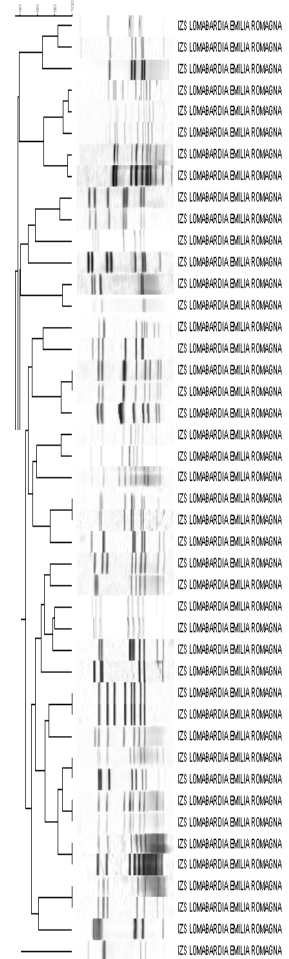


IZS PIEMONTE LIGURIA VALLE D'AOSTA



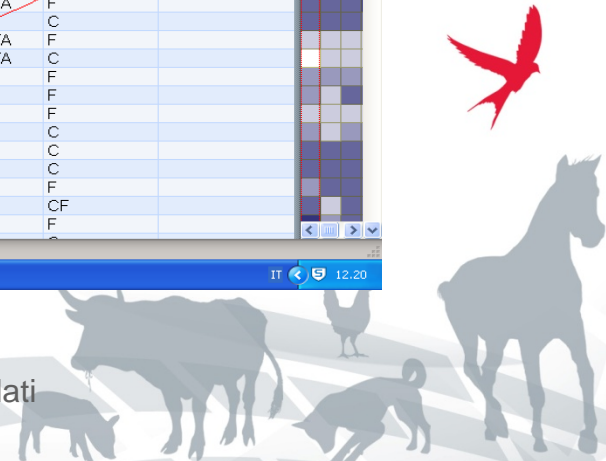
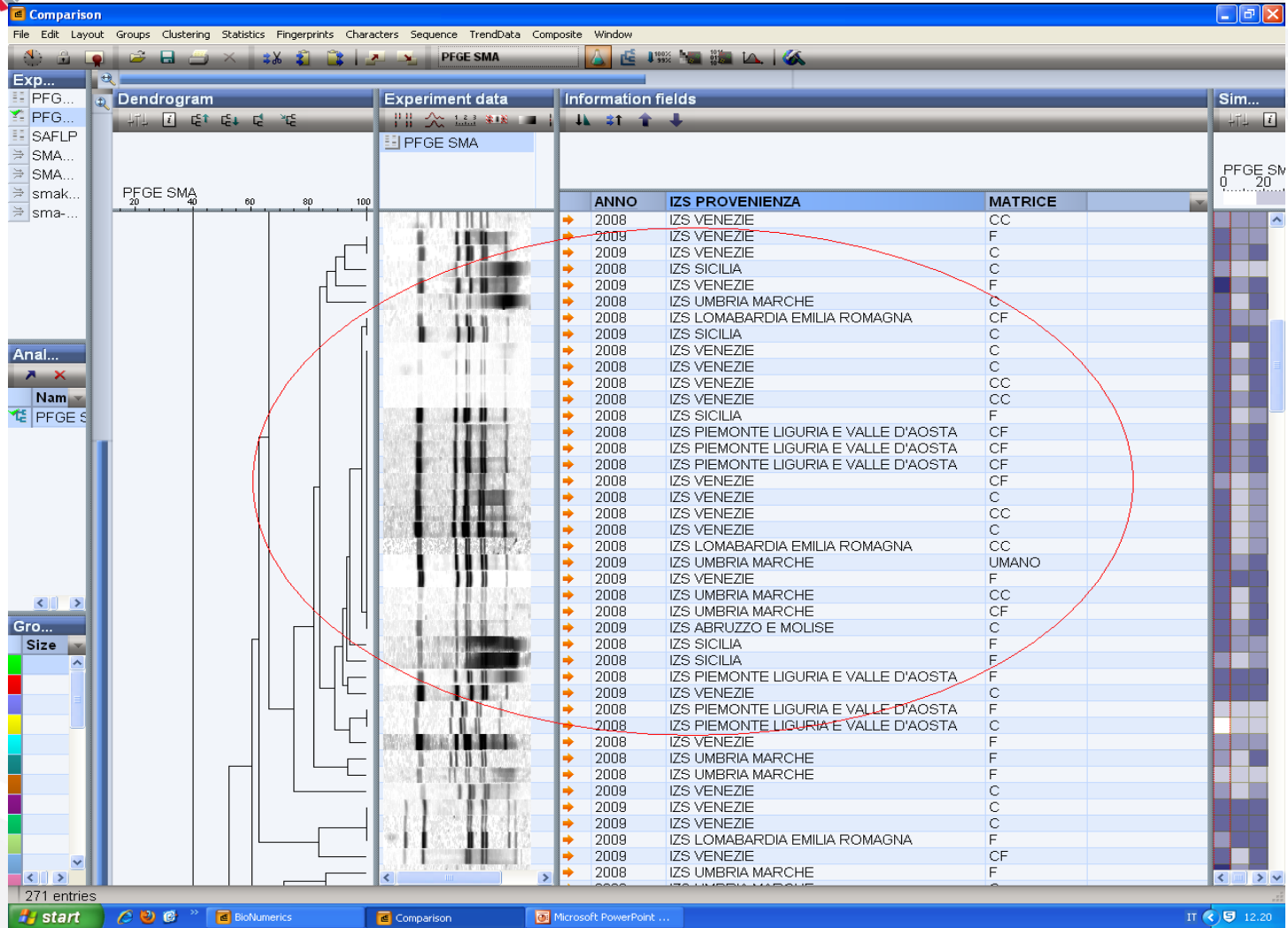
IZS LOMBARDIA EMILIA ROMAGNA

PFGE ID: PFGE SNA

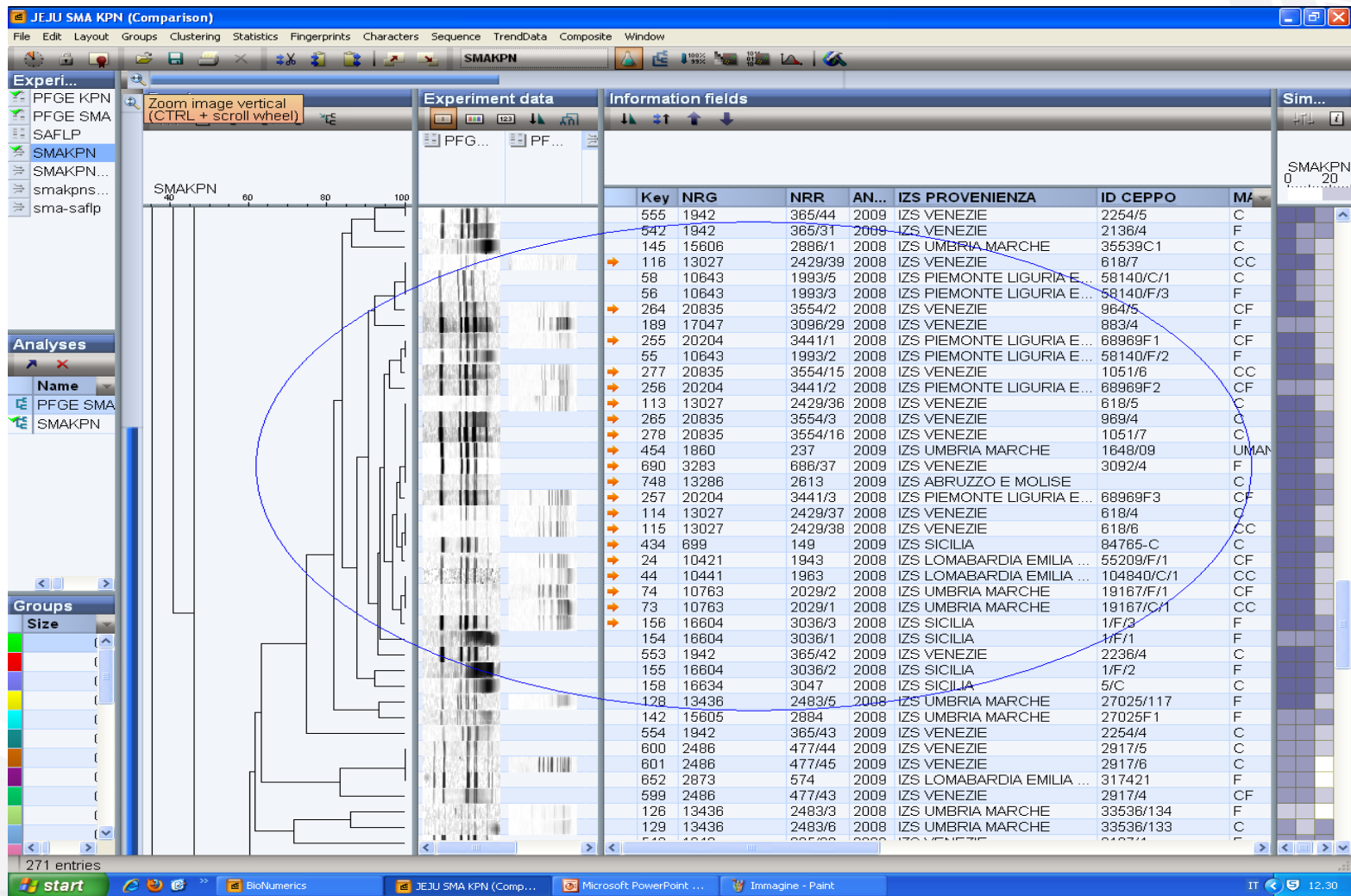




RISULTATI C. JEJUNI

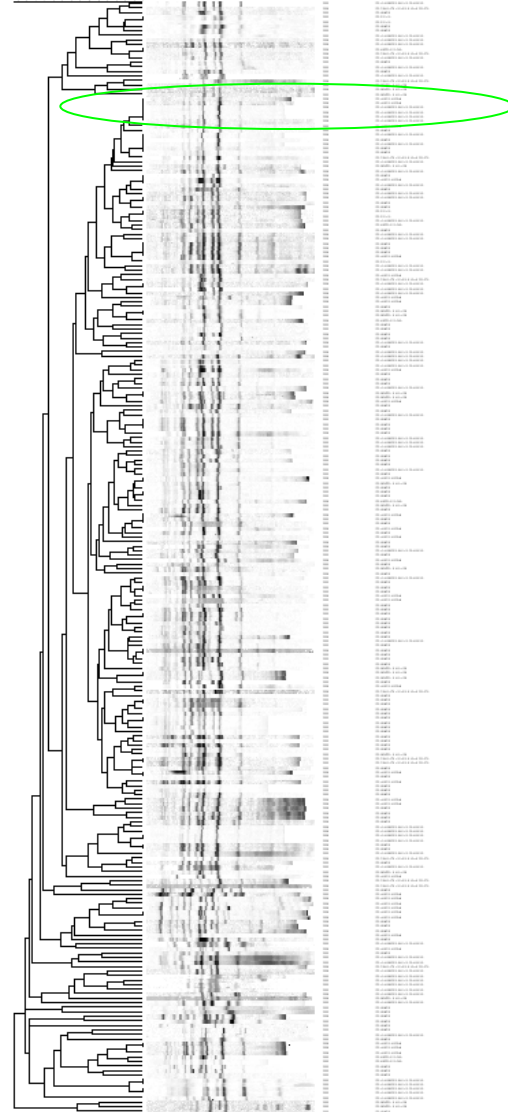
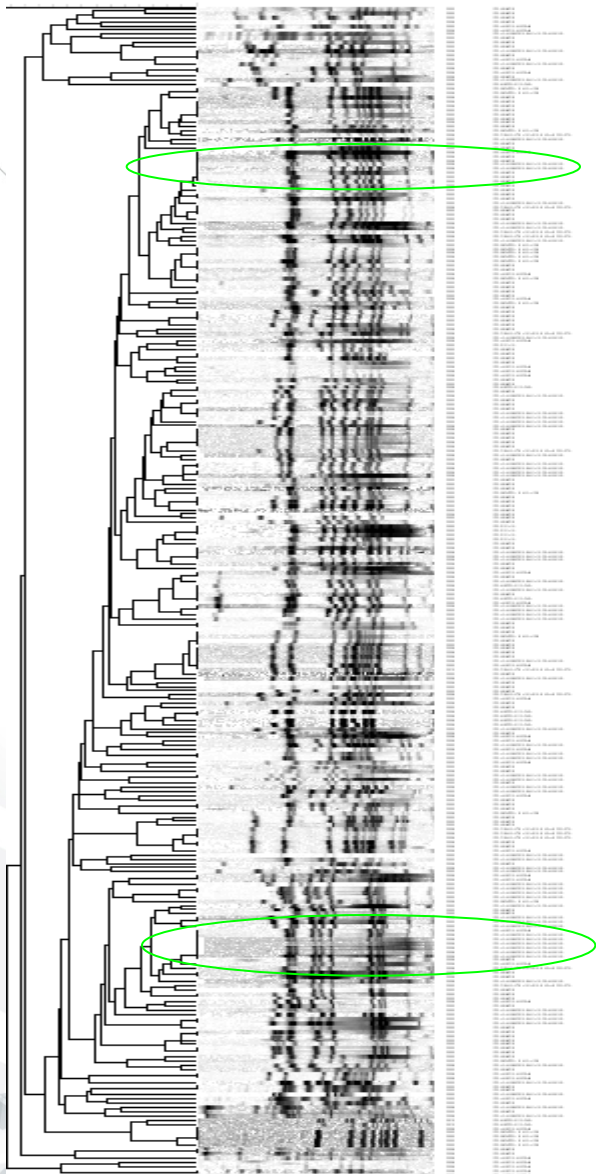


RISULTATI C. JEJUNI



RISULTATI C. COLI

S-AFLP

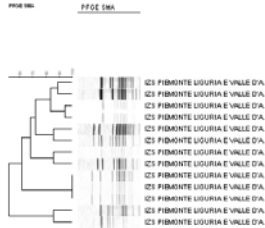




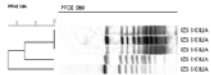
ISTITUTO G. CAPORALE
TERAMO

RISULTATI C. COLI

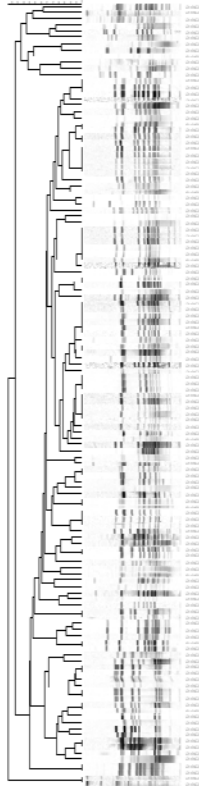
IZS PIEMONTE LIGURIA VALLE D'AOSTA



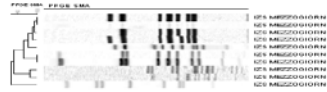
IZS SICILIA



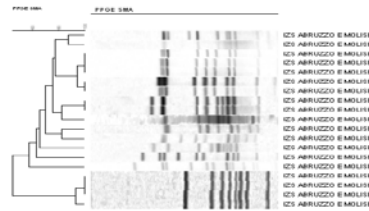
IZS VENEZIE



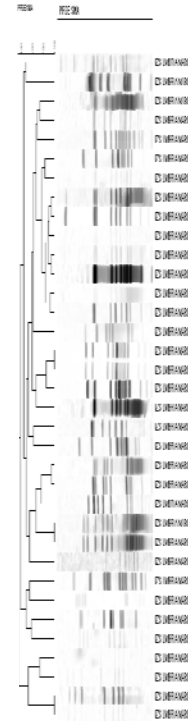
IZS MEZZOGIORNO



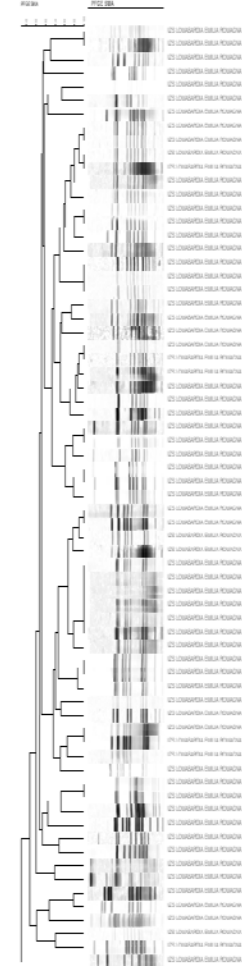
IZS ABRUZZO MOLISE



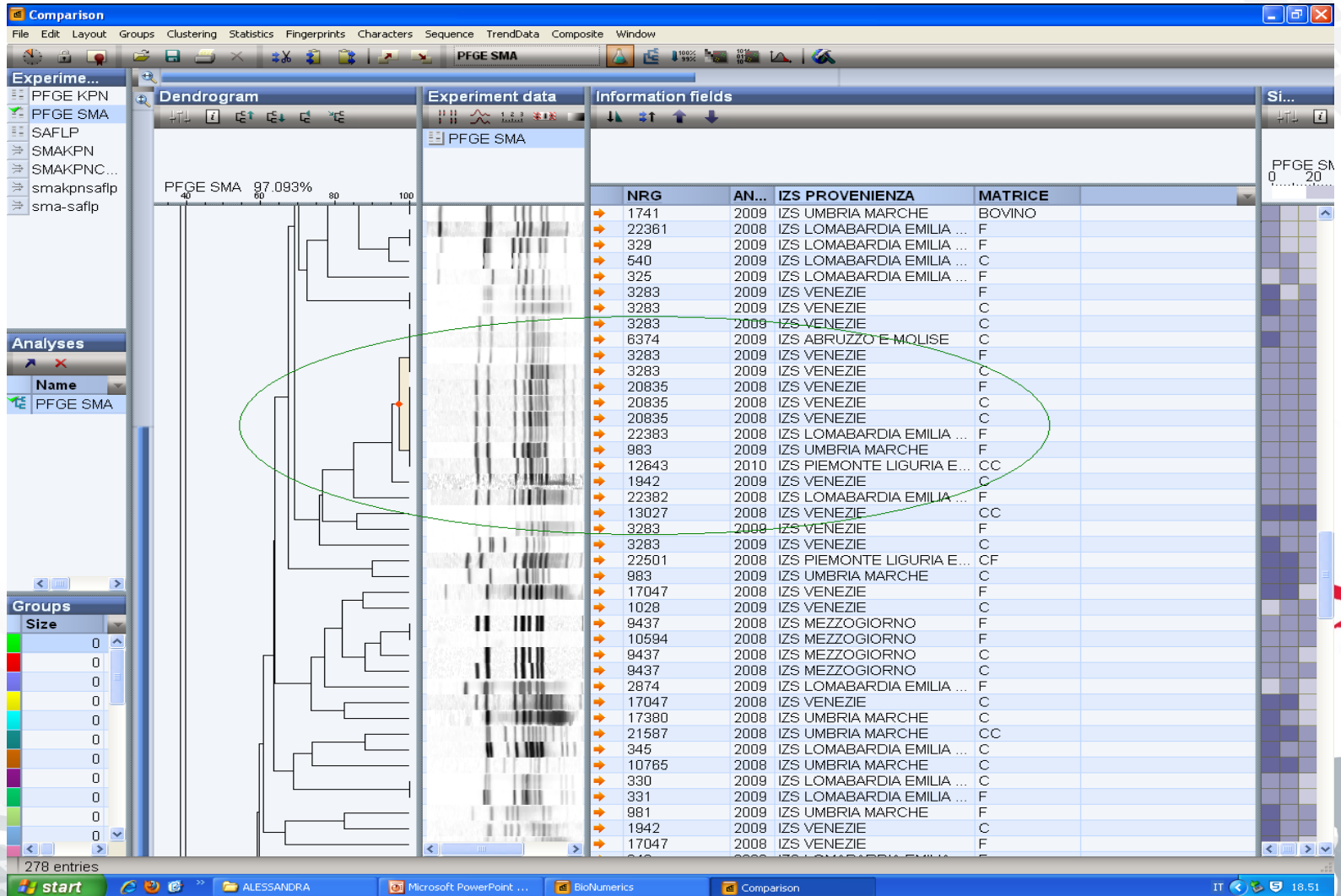
IZS UMBRIA MARCHE



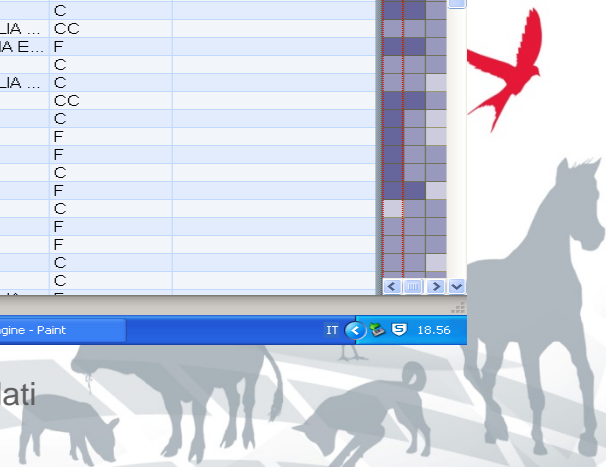
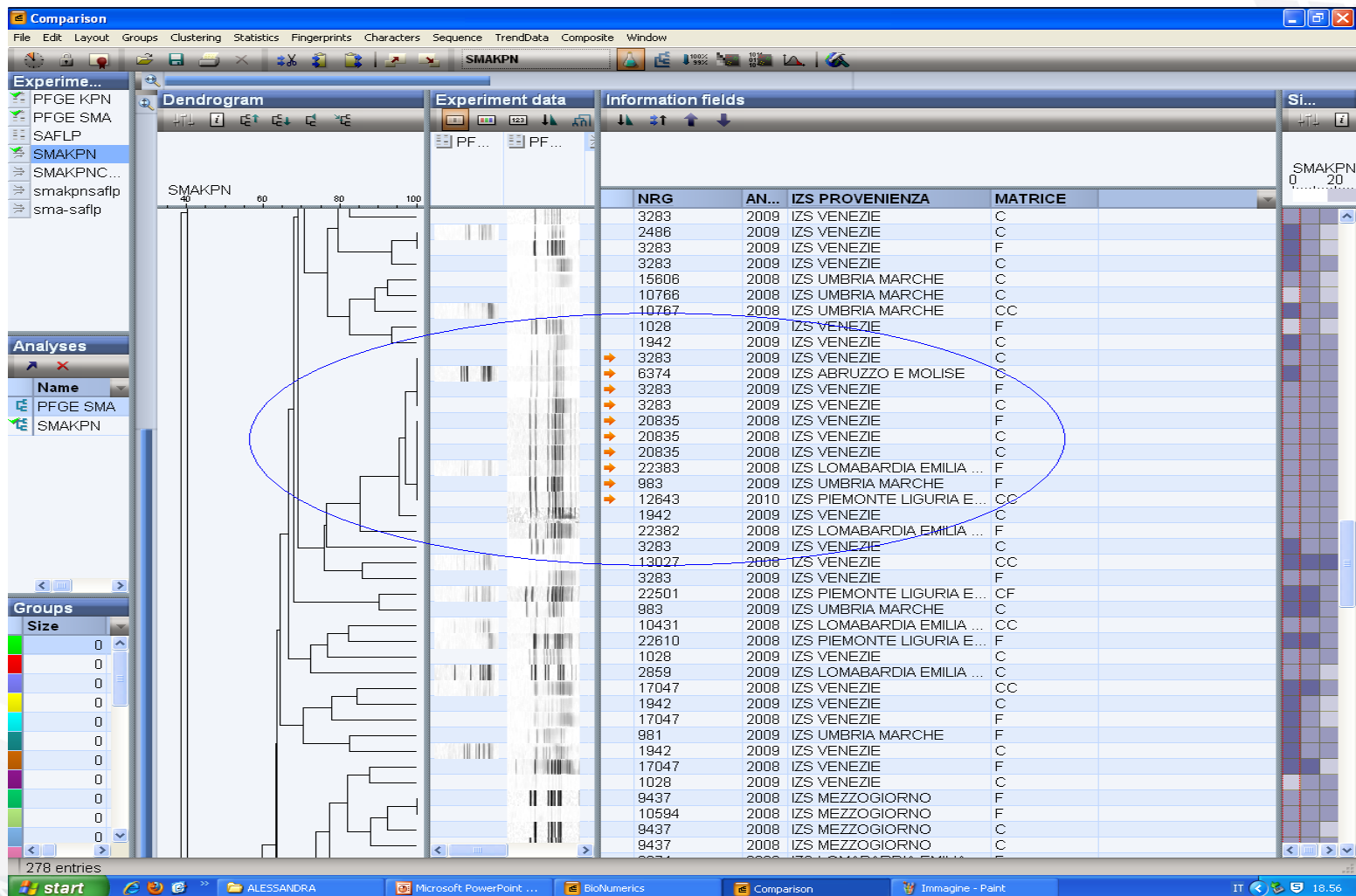
IZS LOMBARDIA EMILIA ROMAGNA



RISULTATI C. COLI



RISULTATI C. COLI



Concludendo:

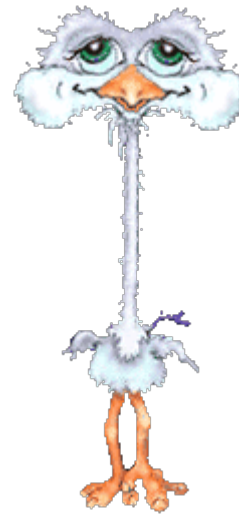
- Esistono popolazioni maggiormente diffuse sul territorio in termini di spazio e tempo sia per *C. jejuni* che per *C.coli*.
- L'antibioticoresistenza ai chinoloni si sviluppa principalmente per mutazione al sito T86-I.





ISTITUTO G. CAPORALE
TERAMO

Grazie per l'attenzione



Baseline study 2008: caratterizzazione dei ceppi isolati
Alessandra Alessiani

