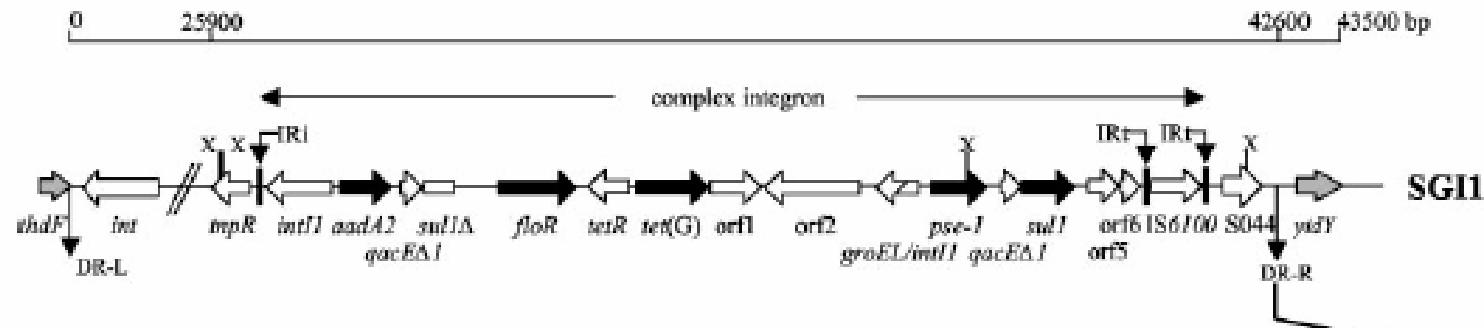


WP21 - Molecular epidemiology of Salmonella Genomic Island 1 (SGI)

Dik Mevius, John Threlfall, Corinne
Amar, Ernesto Liebana, Beatriz
Guerra, Bela Nagy and the WP21
Project team

- April 2006,
 - initially planned until April 2008,
 - extended until April 2009
 - Partners (13):
 - CIDC, ASG (NL)
 - HPA, VLA (UK)
 - AFSSA (F)
 - DFVF, SSI (DK)
 - BFR (G)
 - ISS (I)
 - UCM, ISCIII (S)
 - PZH (P)
 - VMRI (H)
 - Budget: 280 K€ (400 k€)
-
-

- Not to build just a network and exchange expertise obtained locally
- But..
 - To use the funds in an optimum way by
 - Sending isolates selected at partner institutes to HPA
 - Analysis of isolates by a full time molecular biologist
 - Share the expertise obtained at HPA
 - Training course
 - Distribute funds in the last years to stimulate research locally
 - » To optimize output



Frame genes: *thdF* and *int2* (retronphage) in *S. Typhimurium*
thdF and *yidY* in other *S. enterica* serovars

Also flanking the SGI there are 2 direct repeat regions (left and right) DR-L and DR-R

The antimicrobial resistance genes are located near the 3' end:

Complex class 1 integron composed of 2 integrons flanked by inverted repeats IRi and IRt.

Resistance genes: *aadA2*: streptomycin, spectinomycin
bla^{CARB-2} (*pse-1*): ampicillin
floR: chloramphenicol, florfenicol
tetR, *tet(G)*: tetracycline
sul1: sulfonamides

- SGI1 detected in epidemic strains/clones of Salmonella
 - S. Typhimurium Definitive Type (DT) 104
 - Globally one of the predominant ST phagetypes since the 1990s
 - Agona, Cerro, Dusseldorf, Infantis, Kiambu, Typhimurium, Java.
 - Indicative of horizontal spread or a biological reservoir
 - Associated with enhanced virulence
 - Zero tolerance policy exists for DT104
 - Fits in criteria for MVN, covers the four thematic areas:
 - Zoonotic aspects are important
 - Detection&Control, Epidemiology, HP interaction and Risk research
- Because of the limited budget we focus on molecular epidemiology

- This project aims to study the distribution and characteristics of SGI1 in enteric bacteria (a.o. *Salmonella*, *Shigella*, *E. coli*) in a large collection of animal and human isolates. The result will be a database of strains harbouring (parts of) SGI1. This database will be a basis for future virulotyping, and risk assessment of isolates harbouring SGI1.
-
-

Project month	3	6	9	12	15	18	21	24	27	30	33	36
Task												
1. Phenotypic selection method												
2a. PCR protocol for detection of SGI1												
2b. Protocol for molecular analysis of SGI1												
3. Phenotypic selection of Salmonella isolates												
4. Phenotypical selection of other <i>Enterobacteriaceae</i>												
5a Molecular detection of SGI-1 at HPA												
5b. Molecular characterisation of SGI-1, MDR region at HPA												
5c. Protocol for molecular analysis of non-MDR region of SGI-1												
6. Training programme at HPA												
7a Molecular characterisation of non-MDR region at HPA												
7b Selection and molecular analysis at partner institutes												
6. Compilation of the results in a database												

- Based on MDR Phenotype
 - App. 50 Salmonella and 50 E. coli
 - R to Amp/amox, Tet/dox, Sul, Strep/spec, Flor/chlor
 - Exclude DT104
-
-

- 10 participants
 - 399 STRAINS
 - *Salmonella* 276 (28 serovars)
 - *E.coli* 116
 - *Shigella* 7
 - Added some *Shigella* and *Proteus* spp.
 - Collected between 2001-2006
 - Sent between 16.05.2006 and 07.08.2006
 - Source: Human, food, animal
-
-

Results phenotypic selection

	DFVF	SSI	BFR	VMRI	CIDC	UCM	AFSSA	ISS	PZH	VLA	Total
S. Typhimurium		24	11	18	43		2	38		49	185
<i>E. coli</i>	4			17	40	8			2	44	115
S. Enteritidis					1				11		12
S. Infantis				4				6	1		11
S. Saint Paul			3		1		2		2		8
<i>Salmonella</i> spp.			2					5	1		8
S. Derby					4	1					5
S. Newport		4					1				5
Shigella flexneri									5		5
S. Albany			1		1		2				4
<i>S. enterica</i>					4						4
S. Paratyphi B			1		2				1		4
S. Hadar									3		3
S. Heidelberg			2						1		3
S. Mbandaka					3						3
S. Agona					2						2
S. Senftenberg					2						2
S. Thompson									2		2
Shigella sonnei									2		2
S. Brandenburg			1								1
S. Bredeney							1				1
S. Choleraesuis									1		1
S. Cubana					1						1
S. Indiana							1				1
S. Infantis?				1							1
S. Java										1	1
S. Kentucky									1		1
S. Livingstone			1								1
S. Oranienburg									1		1
S. Panama					1						1
S. Rissen					1						1
S. Stanley		1									1
S. Typhi		1									1
S. Wien						1					1
S. Worthington	1										1
Total	5	30	22	40	106	10	9	49	34	94	399

Detection of SGI-1 by gel-based PCRs left and right Junctions

Region detected	Primer	Sequence 5'-->3'	Locus (BLAST searches)	Position in AF261825
Left junction of SGI-1	U7112	ACACCTTGAGCAGGGCAAAG	<i>ThdF</i> S. Typhimurium AF261825 <i>TrmE</i> S. Typhimurium AE008879 LT2 <i>TrmE</i> S. Cholerasuis AE017220	1-20
	LJ-R1	AGTTCTAAAGGTTCTAGTCG	<i>Int</i> S. Typhimurium AF261825	480 -500
Rigth Junction of SGI-1 and retronphage element	104-RJ	TGACGAGCTGAAGCGAATTG	SO44 (protein) S. Typhimurium AF261825	42373-42392
	C9-L2	AGCAAGTGTGCGTAATTTGG	<i>Int2</i> S. Typhimurium AF261825 STM3844 S. Typhimurium AE008879 LT2	42868-42887
Rigth Junction of SGI-1	104-RJ	<i>idem</i> than above		
	104-D	ACCAGGGCAAAACTACACAG	<i>YidY</i> S. Typhimurium, AF261825 <i>YidY</i> S. Typhimurium AE008879 LT2 <i>YidY</i> S Typhi strain AL627280 <i>YidY</i> S. paratyphy A., CP000026 <i>YidY</i> S. Typhi Ty2, AE014613 <i>YidY</i> S. Cholerasuis AE017220	47111-47130

Keys

ThdF thiopene, furan
oxidation

Int integrase

STM3844 integrase

YidY translocase

Detection of Class 1 integrons

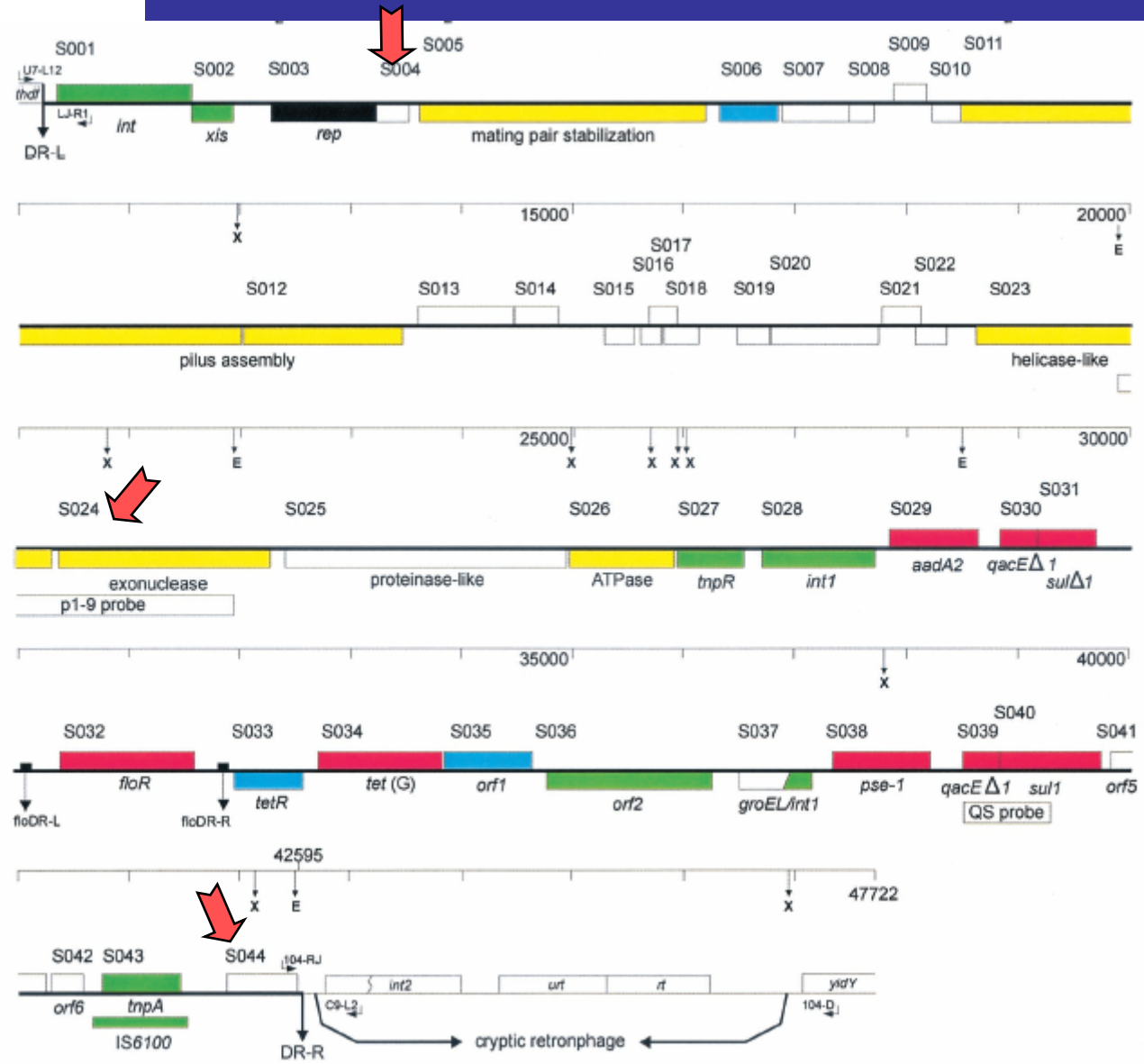
Region detected	Primer	Sequence 5'-->3'	Locus (BLAST searches)	Position in AF261825
Class 1 Integron	L1	GGCATCCAAGCAGCAAG	5'conserved region of Class 1 integron in various Gram negative bacterial species including: <i>Salmonella enterica</i> ; <i>Escherichia coli</i> , <i>Shigella flexneri</i> <i>Pseudomonas aeruginosa</i> , <i>Klebsiella pneumoniae</i> , <i>Campylobacter jejuni</i> , <i>Proteus mirabilis</i> , <i>Morganella morganii</i>	27892-27908 and 37164-37180
	R1	AAGCAGACTTGACCTGAT	3'conserved region of Class 1 integron in various Gram negative bacterial species including: <i>Salmonella enterica</i> ; <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , <i>P. putida</i> , <i>Acinetobacter baumannii</i> , <i>Klebsiella pneumoniae</i> , <i>Citrobacter freundii</i> , <i>Corynebacterium</i> , <i>Proteus mirabilis</i> , <i>Morganella morganii</i> , <i>Campylobacter jejuni</i> ,	28883-28900 and 38343-38360

Phagetyping results:

Typhimurium, SGI +ve n= 128	
PT	Total
12	3
92	1
104	2
120	1
151	1
193	2
104b	32
104C	4
104L	44
12 L	1
12(L)	1
120 Rough I:1.2	4
206 var	2
DT104	1
U302	18
U302 L	1
U309	2
UNTYP	2
UNTYP I:1.2	1
RDNC	5
Grand Total	128

Development of real time assays for the detection of SGI-1: upstream and downstream the MDR region.

- Detection of the SGI-1 protein gene fragments.
 - S004
 - S024 (P1.9 probe hybridization region)
 - S044



RESULTS

Strains SGI POSITIVE by gel-based PCR	n =	Taqman assays results		
		S004 pos	S024 pos	S044 pos
S. Albany	3	3	3	3
S. Derby	2	2	2	2
<i>S. enterica</i>	1	1	1	1
S. Java	1	1	1	1
S. Kentucky	1	1	1	1
S. Newport	4	4	4	4
S. Paratyphi B	4	4	4	4
S. Typhimurium	128	128	128	128
Grand Total	144	144	144	144

Strains LEFT Junction only positive by gel-based PCR	n =	S004 pos	S024 pos	S044 pos
<i>S. Typhimurium</i>	1	1	1	0
Strains SGI NEGATIVE by gel-based PCR				
	n =	S004 pos	S024 pos	S044 pos
<i>E. coli</i>	116	0	0	0
<i>S. Agona</i>	1	1	0	0
<i>S. Albany</i>	1	1	1	1
<i>S. Brandenburg</i>	1	0	0	0
<i>S. Bredeney</i>	1	0	0	0
<i>S. Choleraesuis</i>	1	0	0	0
<i>S. Cubana</i>	1	0	0	0
<i>S. Derby</i>	4	3	0	0
<i>S. Enteritidis</i>	11	0	0	0
<i>S. Hadar</i>	3	0	0	0
<i>S. Heidelberg</i>	3	0	0	0
<i>S. Indiana</i>	1	0	0	0
<i>S. Infantis</i>	11	0	0	0
<i>S. Livingstone</i>	1	0	0	0
<i>S. Mbandaka</i>	3	0	0	0
<i>S. Newport</i>	1	0	0	0
<i>S. Oranienburg</i>	1	0	0	0
<i>S. Panama</i>	1	0	0	0
<i>S. Rissen</i>	1	0	0	0
<i>S. Saint Paul</i>	8	0	0	0
<i>S. Stanley</i>	1	0	0	0
<i>S. Thompson</i>	2	0	0	0
<i>S. Typhi</i>	1	0	0	0
<i>S. Typhimurium</i>	66	0	0	0
<i>S. Wien</i>	1	0	0	0
<i>S. Worthington</i>	1	0	0	0
Salmonella	4	0	0	0
Shigella flexneri	5	0	0	0
Shigella sonnei	2	0	0	0
Grand Total	254	5	1	1

← L1-R1: 1000+1200

← L1-R1: 1000+1200

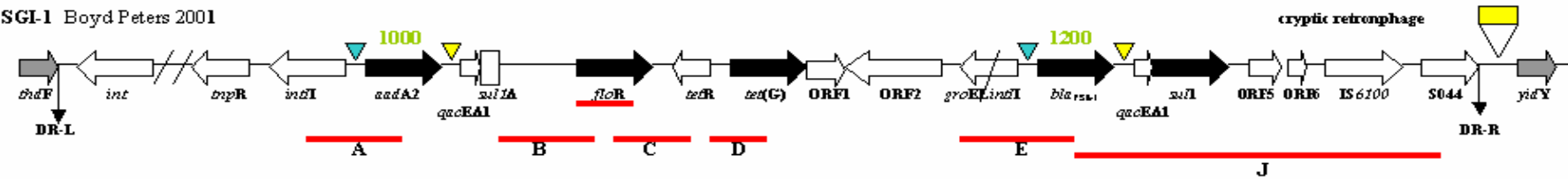
← L1-R1: none

← L1-R1: 1000 only

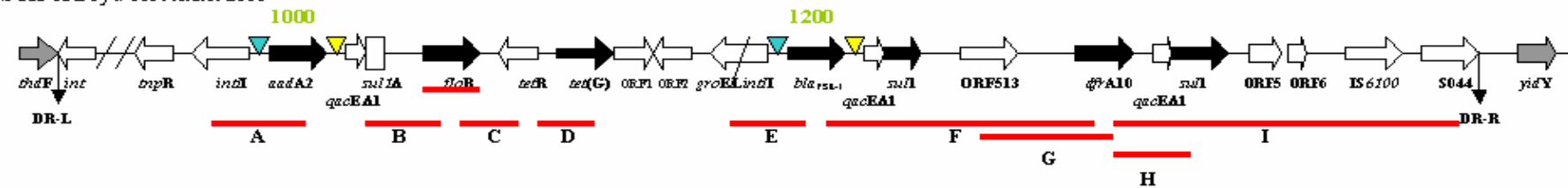
Characterization of the MDR region

I

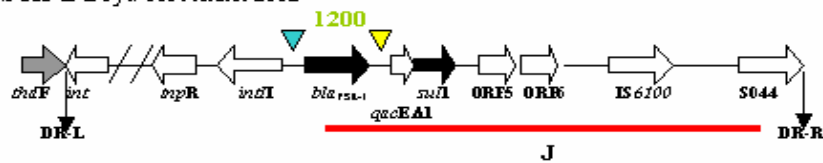
SGL-I Boyd Peters 2001



SGII-A Boyd Cloeckart 2000



SGII-B Boyd Cloeckart 2002



PCR	FLO	A	B	C	D	E	F	G	H	I	J
Length	494	1135	942	598	1559	1338	4748	2144	1269	3791	4407
SIG-1	FLO	A	B	C	D	E					J
SIG-A	FLO	A	B	C	D	E	F	G	H	I	
SIG-B											J
SIG-C		A									
SIG-D		A						G	H	I	
SIG-E											
SIG-F	FLO		B	C	D	E					J
SIG-G							F	G	H	I	
SIG-H	FLO		B	C	D	E					J
SIG-I	FLO	A	B	C	D				H length?	I >3791bp	
SIG-J	FLO		B	C	D						
SIG-K											
SIG-L	FLO		B	C	D	E					J

STRAIN	N=	SGI	L1/R1	SGI-04 CT	SGI-24 CT (p1.9)	SGI-44 CT	Flo PCR 494bp	A PCR 1135 bp	B PCR 942 bp	C PCR 598 bp	D PCR 1559 bp	E PCR 1338 bp	F-PCR 4748 bp	G PCR 2144 bp	H PCR 1269 bp	I PCR 3791 bp	J PCR 4400 bp	MDR SGI
S. Agona	1	+	1000+1200	17	18	17	Flo	A	B	C	D	E		G	H			SGI-A
S. Agona	1	neg	1000+1200	19	neg	neg	Flo	A	B	C	neg	E		neg	neg			
S. Albany	3	+	1300	15	17	16	Flo	neg	B	C	D	E		neg	neg			SGI-F-HL
S. Albany	1	neg	NEG	16	18	16	Flo	neg	neg	C	D	E		neg	neg			
S. Derby	2	neg	1000	16	neg	neg	neg	A	neg	neg	neg	neg		neg	Hw			
S. Derby	1	+	1000+1200	16	18	17	Flo	A	neg	C	D	E		G	H			SGI-A?
S. Derby	1	neg	1000	16	neg	neg	neg	A	neg	neg	neg	neg		neg	neg			SGI-C?
S. Enteritidis	1	+	1000+1200	14	16	15	Flo	A	B	C	D	E		neg	neg			SGI-1
S. Java	1	+	1000+1200	17	22	17	Flo	A	B	C	D	E		neg	H			
S. Kentucky	1	+	1600	15	19	15	neg	neg	neg	neg	neg	neg		neg	neg			
S. Newport	3	+	750+1200+1600	17	18	17	Flo	neg	B	C	D	E		neg	neg			SGI-F-HL
S. Newport	1	+	750+1200+1600	15	16	16	Flo	neg	neg	C	D	E		neg	neg			
S. Senftenberg	2	+	1000+1200	17	18	17	Flo	A	B	C	D	E		neg	neg			SGI-1
S. Typhimurium	61	+	1000+1200	20	21	20	Flo	A	B	C	D	E		neg	neg			SGI-1
S. Typhimurium	24	+	1000+1200	14	16	14	Flo	A	neg	C	D	E		neg	neg			SGI-1?
S. Typhimurium	16	+	1000+1200	26	21	25	Flo	A	neg	C	neg	E		neg	neg			
S. Typhimurium	14	+	1000+1200	22	21	19	Flo	A	B	C	neg	E		neg	neg			SGI-1?
S. Typhimurium	1	Left only	1000+1200	15	17	neg	Flo	A	B	C	neg	E		neg	neg			SGI-1?
S. Typhimurium	1	+	1000+1200+1600	14	15	15	Flo	A	B	C	neg	E		neg	neg			SGI-1?
S. Typhimurium	1	+	1000+1200+1600	17	18	18	Flo	A	neg	C	D	E		neg	neg			SGI-1?
S. Typhimurium	1	+	1200	16	17	16	neg	neg	neg	neg	neg	neg		neg	neg			SGI-B?

- Very complex results.
- Time consuming
- PCR amplifications are not consistent, which means a lot of repeats
- Detection of extra-chromosomal sequences.
- New variants still to be identified.

Not ideal for large scale screening



- Odd strains:
 - Truncated SGI?
 - Polymorphism?
 - Obtained amplicons: Chromosomal or on plasmid?

Truncated SGI?

- Inverse PCR
 - Digest the genome.
 - Ligate each fragments into ‘circles’.
 - One type of ‘circle’ will have a ‘known’ and an unknown region.
 - Perform inverse PCR: 2 primers (from the known region) going in opposite directions in the circle.
 - Amplify.
 - Sequence.

Fingerprinting of the SGI-1

- 95 pairs of primers have been designed
- The amplicons span the SGI-1
- Approximately every 500bp
- Strains to test:
 - Odd strains
 - Known positive *S. Typhimurium*
 - *S. Non Typhimurium*, SGI-1 positive
 - A few *Proteus* and *Shigella* strains

- Strain collection of SGI1 + isolates
 - Molecular characterisation
 - SGI-types based on
 - MDR region
 - Class I integron present
 - Odd isolates with truncated SGI1 detected
 - All SGI1 + isolates are virulotyped
 - A training course on molecular analysis of MDR anticipated
 - 1 publication in preparation, 1 or 2 anticipated on work done at HPA
 - Several posters presented
 - Spinn-off publications and posters on research done at partner institutes
 - A strong network created
 - FP7 application in preparation
-
-

Acknowledgment

- All participants of the WP21 for the donation of the strains
- Elizabeth De Pinna and colleagues from LEP for the phagetyping, antibiotic resistance tests and serotyping.
- Tom Cheasty from LEP for donation of Shigella strains
- Neil Woodford from ARMRL for the donation of Proteus strains
- Paul Dear from the Laboratory of Molecular Biology, MRC Cambridge for support and advice.