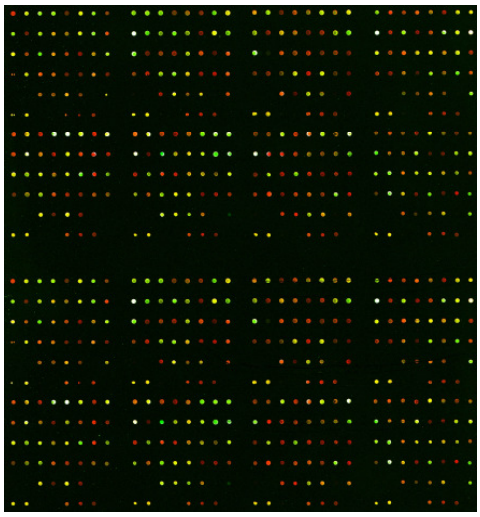
Development and validation of a DNA microarray for characterisation and typing of *Salmonella* isolates



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Meinen Eltern

Abstract

Poultry is recognised as an important source for human infections caused by *Salmonella enterica* subsp. *enterica*. A European baseline survey during the years 2005 and 2006 has revealed that the monophasic *Salmonella enterica* subsp. *enterica* serovar 4,12:d:- and the serovar Paratyphi B d-tartrate positive (dT+) were one of the most frequently isolated serovars in German broiler flocks.

This study focuses on the genotypic characterisation of these both serovars using pulsed-field gel electrophoresis and DNA microarray to determine the clonality, the pathogenic gene repertoire, and resistance determinants. For that purpose, a prototype of a *Salmonella* DNA microarray comprising 276 60-mer and 5 40-mer oligonucleotide probes was developed and in-house validated.

Nearly identical PFGE profiles and a highly similar gene repertoire were found among serovar 4,12:d:- isolates of feed, animal and human sources. All strains were susceptible to 16 antimicrobial agents tested and did not encode any resistance genes. The serovar lacked genes with known contributions to pathogenicity in comparison to serovars highly prevalent in humans. The comparison of the virulence gene repertoire to other serovars indicated the highest relationship to serovar Derby (4,12:f,g:[1,2]). Among serovar Paratyphi B dT+ isolates two major clonal lines, which could be phenotypically differentiated by the expression of the O:5-antigen, were identified. All O:5 antigen negative (O:5-) strains were multi-drug resistant and originated from Western Europe (Belgium, Netherlands and Germany) poultry. Strains exhibiting the O:5-antigen (O:5+) encoded by the *oafA* gene revealed a more heterogeneous group including multi-drug resistant and susceptible strains. Compared to O:5- isolates, serovar Paratyphi B dT+ O:5+ strains possessed additional virulence determinants. The *Salmonella* Genomic Island 1 was only found in O:5+ strains. Five monophasic serovar 4,5,12:b:- isolates lacking the phase-2 flagellar antigen were highly similar to serovar Paratyphi B dT+ isolates of the O:5+ group.

Currently it can not be estimated that serovar 4,12:d:- exposes a high risk to humans caused by consumption of poultry. In concern of serovar Paratyphi B dT+ a multi-drug resistant clone still persists in chickens across Western Europe. Moreover, the existence of a second, more heterogeneous serovar Paratyphi B dT+ O:5+ group was shown encoding additional fimbrial and virulence genes suggesting a more diverse origin and an ubiquitous spreading.

Zusammenfassung

Geflügel stellt eine wichtige Quelle für durch *Salmonella enterica* subsp. *enterica* verursachte Lebensmittel assoziierte Humaninfektionen dar. Das in den Jahren 2005 und 2006 durchgeführte Europäische Masthähnchen Monitoring zeigte, dass der monophasische *Salmonella enterica* subsp. *enterica* Serovar 4,12:d:- und der Serovar Paratyphi B, d-Tartrat fermentierend (dT+), die am häufigsten isolierten Serovare aus Masthähnchen Herden waren.

Die in dieser Studie durchgeführte genotypische Charakterisierung untersucht diese beiden Serovare hinsichtlich ihrer Klonalität, dem Pathogenitätsgenrepertoire und Resistenzdeterminanten mittels Pulsfeld-Gelelektrophorese und DNA Microarray. Zu diesem Zweck wurde ein neuer Prototyp eines *Salmonella* DNA Microarrays, ausgestattet mit 276 60-mer und 5 40-mer Oligonucleotiden, entwickelt und validiert.

Nahezu identische PFGE Profile und ein sehr ähnliches Genrepertoire wurden für Serovar 4,12:d:- Isolate aus Futtermittel, Tier und Mensch gefunden. Alle Stämme waren sensibel gegenüber den 16 getesteten Antibiotika und kodierten keine Resistenzgene. Dem Serovar fehlten Pathogenitätsgene, die üblicherweise von häufig in Menschen vorkommenden Serovaren kodiert werden. Der Vergleich des Virulenzgenrepertoires mit anderen untersuchten Serovaren zeigte die höchste Verwandschaft zum Serovar Derby (4,12:f,g:[1,2]). Serovar Paratyphi B dT+ Isolate unterteilten sich in zwei klonale Linien, die phänotypisch durch die Expression des O:5 Antigens unterschieden werden konnten. Alle aus Westeuropa (Belgien, Niederlande und Deutschland) stammenden O:5 negativen (O:5-) Stämme waren multiresistent und aus Geflügel isoliert. Stämme die über das O:5 Antigen verfügten (O:5+), kodiert durch *oafA*, zeigten eine heterogene Gruppe die sowohl sensible, als auch resistente Stämme einschloss. Im Vergleich zu den O:5- Isolaten, besaßen O:5+ Stämme zusätzliche Virulenzdeterminanten. Die *Salmonella* Genomic Island 1 wurde nur in den O:5+ Stämmen gefunden. Fünf monophasische Serovar 4,5,12:b:- Stämme, denen das Phase2-Antigen fehlte, waren den Serovar Paratyphi B dT+ Isolaten der O:5+ Gruppe sehr ähnlich.

Zur Zeit kann nicht davon ausgegangen werden, dass Serovar 4,12:d:- ein hohes Risiko beinhaltet, nach Verzehr von Geflügel Humaninfektionen auszulösen. Für Paratyphi B dT+ O:5- konnte die Persistenz eines multiresistenten Klones in Westeuropa in Geflügelbeständen nachgewiesen werden. Weiterhin wurde die Existenz einer zweiten, heterogenen O:5+ Serovar Paratyphi B dT+ Gruppe nachgewiesen, die zusätzliche Fimbrien- und Virulenzgene kodiert und sehr wahrscheinlich ubiquitär verbreitet ist.

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List of Abbreviations-

-	negative relative intensity signal of a gene
+	positive relative intensity signal of a gene
AMC	Amoxicillin/Clavulanic acid
AMP	Ampicillin
A+T	Adenosine and Thymidine
BfR	Federal Institute for Risk Assessment, Berlin, Germany
bp	base pairs
CFU	colony forming units
CHL	Chloramphenicol
CIP	Ciprofloxacin
CLSI	Clinical Laboratory Standards Institute
COL	Colistine
C+G	Cytosine and Guanosine
DNA	deoxyribonucleic acid
dNTP	deoxy-nucleotide triphosphate
DT	definitive type, phage type of lysotyping of *S. enterica* serovar Typhimurium
DTU	Technical University of Denmark
dT+	dextrorotatory [L+]-tartrate fermenting
EDTA	ethylene diamine tetra-acetic acid
ESBL	Extended spectrum β-Lactamase
FLO	Florfenicol
GAL	GenePix Array List
GALT	gut-associated lymphoid tissue
HPLC	high performance liquid chromatography
IHC	Internal Hybridisation Control
Inc	Incompatibility group
IS	Insertion Sequence
KAN	Kanamycin
kb	kilobases
LB-medium	Luria-Bertani-Medium

LPS	lipopolysaccharide
MALDI	matrix assisted laser desorption ionisation (mass spectrometry)
MDR	multi-drug resistance
MIC	Minimum Inhibitory Concentration
NAL	Nalidixic acid
NEO	Neomycin
no.	number
NRL-Salm	National *Salmonella* Reference Laboratory
NT	non-typable
PBS	phosphate buffered saline
PC	Personal Computer
PFGE	Pulsed-Field Gel Electrophoresis
PMT	Photomultiplier
PT	phage type of lysotyping of *S. enterica* serovar Enteritidis
PTS	phosphotransferase-system
RKI	Robert Koch Institute
RT	room temperature
RNA	ribonucleic acid
rpm	rounds per minute
S. enterica	*Salmonella enterica*
s.a.	see above
SDE	*Salmonella enterica* serovar Derby
SDU	*Salmonella enterica* serovar Duisburg
SDS	sodium-dodecyl-sulfate
SEN	*Salmonella enterica* serovar Enteritidis
SePop	Selective Puritifaction optimized process
SGI-1	*Salmonella* Genomic Island 1
SIN	*Salmonella enterica* serovar Infantis
SLI	*Salmonella enterica* serovar Livingstone
SMX/SUL	Sulfamethoxazole
SNP	Single Nucleotide Polymorphism
SPB dT+	*Salmonella enterica* serovar Paratyphi B dT+
SPE	Spectinomycin
spp.	Subspecies

SST	*Salmonella enterica* serovar Stanley
SSW	*Salmonella enterica* serovar Schwarzengrund
STM	*Salmonella enterica* serovar Typhimurium
STR	Streptomycin
subsp.	Subspecies
SUO	*Salmonella* strain from the University of Oviedo
SXT	Sulfamethoxazole/Trimethoprim
TET	Tetracycline
T_m	melting temperature
TMP	Trimethoprim
Tn	Transposon
Tris	Tri(hydroxymethyl)-amino methane
UPGMA	unweighted-pair group method with arithmetic averages
VLA	Veterinary Laboratories Agency
WHO	World Health Organisation

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1. Introduction

Infections by *Salmonella enterica* are a significant public health concern around the world. *Salmonella* infections are the second leading cause of bacterial food borne illness in the United States and Europe (62,159). An estimated 95% of these salmonellosis cases are associated with the consumption of contaminated food products (161). The impact of *Salmonella* infections on the economy in the United States has been estimated at approximately $3.6 billion due to loss of work, medical care and loss of life (79). Based on such economic impact and statistics there is a worldwide interest in lowering *Salmonella* infections. Within the last decade a number of various phenotypic and genotypic methods have been developed to distinguish *Salmonella* from each other to understand their epidemiology, pathogenicity, resistance and spread in animals, humans and their environment. Newer approaches like DNA microarrays enable the characterisation of whole genomes resulting in detailed data that gives the basis for estimating the potential hazard of individual strains for humans and animals. In this thesis a DNA microarray was developed as a tool to characterise *Salmonella* serovars and to apply the microarray to two serovars which are known to be associated with poultry. The data were used to discuss the potential hazard for humans.

1.1 *Salmonella* taxonomy and serological classification

The genus *Salmonella* is a member of the *Enterobacteriaceae* family; they are rod-shaped facultative anaerobic, catalase-positive, oxidase-negative, gram-negative bacteria which are regularly motile. The bacteria are non-spore forming with a size of 0.7-1.5 µm × 2.0-5.0 µm and producing colonies generally 2-4 mm in diameter (130). The genus is named after the American bacteriologist D.E. Salmon who identified *S. choleraesuis* in 1885 (207). Two species are currently recognised, *Salmonella enterica* and *Salmonella bongori* (formerly subspecies V). *S. enterica* has been subdivided into six subspecies (subsp.), *S. enterica* subsp. *enterica* (designated subspecies I), *S. enterica* subsp. *salamae* (subspecies II), *S. enterica* subsp. *arizonae* (subspecies IIIa), *S. enterica* subsp. *diarizonae* (subspecies IIIb), *S. enterica* subsp. *houtenae* (subspecies IV) and *S. enterica* subsp. *indica* (subspecies VI) (85), respectively. Subspecies I strains are usually isolated from humans and warm-blooded animals (85,188). The other subspecies usually originate from cold-blooded animals and the environment (24). A number of biochemical reactions are used to differentiate between the species and subspecies (85) as shown in Table 1.

Table 1: Biochemical characters of *Salmonella* species and subspecies

Species	***S. enterica***						***S. bongori***
Subspecies	*enterica*	*salamae*	*arizonae*	*diarizonae*	*houtenae*	*indica*	
biochemistry							
Dulcitol	+	+	–	–	–	d	+
ONPG (2 h)	–	–	+	+	–	d	+
Malonate	–	+	+	+	–	–	–
Gelatinase	–	+	+	+	+	+	–
Sorbitol	+	+	+	+	+	–	+
Growth with KCN	–	–	–	–	+	–	+
L(+)-tartrate(a)	+	–	–	–	–	–	–
Galacturonate	–	+	–	+	+	+	+
γ-glutamyl-transferase	+(*)	+	–	+	+	+	+
ß-glucuronidase	d	d	–	+	–	d	–
Mucate	+	+	+	– (70%)	–	+	+
Salicine	–	–	–	–	+	–	–
Lactose	–	–	– (75%)	+ (75%)	–	d	–
Lysed by phage O1	+	+	–	+	–	+	d
Usual habitat	Warm-blooded animals		Cold-blooded animals and environment				

(a) Dextro rotatory (L-) tartrate
(*) *S. enterica* serovars Typhimurium (d), Dublin –
\+ 90% or more positive reactions
\- 90% or more negative reactions
d different reactions by different serovars

According to the White-Kauffmann-Le Minor scheme (85), subspecies are further divided into serovars (serotypes) by serologic testing with monovalent antisera. Serotyping is widely used as an epidemiological and standardised typing method for *Salmonella*. Approximately 99% of the 2579 currently known serovars composing the genus belong to *S. enterica* (85). The DNA sequence similarity between serovars is 96-99% (61). Serotyping is based on the antigenic variability at lipopolysaccharide moieties (O-antigen), the phase-1 and phase-2 flagellin (H1- and H2-antigen), as well as for some serovars on the capsular polysaccharide

(Vi-antigen). Altogether 67 different serogroups defined by O-antigens, classified by the White-Kauffmann-Le Minor scheme are known. Serovars are designated by their antigenic formulae (O-antigen:H1-antigen:H2-antigen) following the subspecies name. Serovars belonging to *Salmonella enterica* subspecies I are designated with a name which is related to the geographical place where the serovar was first isolated. (85). For example, the *Salmonella enterica* subsp. *enterica* serovar with the antigenic formula 4,5,12:i:1,2 has the name *Salmonella* Typhimurium.

Serovars can be phenotypically further subtyped by specific phage typing. Human and mammalian animal infections are often caused by few serovars belonging almost exclusively to *S. enterica* subsp. *enterica*. However, other animal classes e.g. reptiles show predominant infections from other subspecies, in this case IIIa (137). These very common *S. enterica* subsp. *enterica* serovars cannot be adequately characterised by serotyping alone. Phage typing enables a detailed subtyping of a certain *Salmonella* serovar. This typing technique infects a strain of a certain serovar with a number of bacteriophages. By this technique a set of defined phages is used to obtain a lysis pattern of the strain. This pattern is associated to a definite phage type (6,35). Prominent phage type systems are available for the serovars Typhimurium (193) and Enteritidis (253). Phage typing has been shown to be very useful in the description of pandemic clones of *Salmonella*, such as *Salmonella* serovar Typhimurium definitive type 104 (DT104) (191).

1.2 Epidemiology

Salmonella is a major zoonotic food-borne pathogen causing outbreaks and sporadic cases of gastroenteritis in humans in Europe and worldwide (111,199). In 2007, a total of 151,995 confirmed cases of human salmonellosis were reported in the European Union (EU) by the European Surveillance System (62). The EU incidence was 31.1 cases per 100,000 inhabitants, ranging in the countries from zero to 171.6 cases per 100,000. This represents a decrease of 7.3% compared to 2006, despite new EU member countries (Romania and Bulgaria) and a 16.6% decrease compared to 2005. In the United States approximately 168,000 physician office visits, 15,000 hospitalisations and 400 deaths owing to salmonellosis are reported annually (249). It is estimated that the real number of infections exceeds this tenfold due to misdiagnosis and underreporting of gastrointestinal illnesses. The most epidemiological important serovars *S. enterica* serovar Enteritidis and *S. enterica* serovar Typhimurium are responsible for 79% of all human infections worldwide (263). In 2007, *S. enterica* serovar Enteritidis was implicated in 64.5% of cases of human salmonellosis in

Europe (62). Other most frequently isolated *S. enterica* serovars are Typhimurium (16.5%), Infantis (1%), Virchow (0.8%), Newport (0.6%), Stanley (0.5%), Hadar (0.4%), Derby (0.4%), Kentucky (0.3%), and Agona (0.3%) (62). However, the prevalence of certain serovars varies between the countries. A transmission cycle of *Salmonella enterica* between environmental sources, vegetable food- or animal-feed plants, animals, food and humans, has been recognised (23,50,53,120). Human salmonellosis is mainly caused by ingestion of contaminated food. In turn, contaminated animal feed and wild life animals (e.g. birds) have been recognised as important entry sites in the food chain in farm livestock (220).

Over 98% of registered *Salmonella* infections of mammalian and birds belong to subspecies I (171). The last two percent belongs mostly to subspecies IIIa and IIIb. Salmonellae of subspecies II and IV are often associated with reptile infections (24). Certain serovars of *S. enterica* are host-adapted and cause systemic disease. Human host-adapted *S. enterica* serovars Typhi, Paratyphi A, Paratyphi B (dT-), Paratyphi C may predominantly cause typhoid fever (28,60,121). Other species adapted serovars are *S. enterica* serovars Dublin and Choleraesuis that are generally associated with severe systemic disease in ruminants and pigs, respectively (42,84). *S. enterica* serovars Gallinarum and Abortusovis are almost exclusively associated with systemic disease in fowl (264), and sheep (179), respectively.

The prevalence of *Salmonella* in food-producing animals differs widely amongst the species. Several European baseline surveys performed by each EU-member state within recent years gave a reliable estimation on the prevalence of *Salmonella* in food-producing animals (7). It was observed that the *Salmonella* prevalence and serovar distribution can vary widely among the EU-Member States. In flocks of turkeys the *Salmonella* prevalence was 10.3% in Germany. The three most frequently serovars were Typhimurium (25.8%), Saintpaul (16.1%) and Hadar (12.9%) (67). In 2005, for laying hens the prevalence of *Salmonella* positive holdings were 28.9%. The most frequently isolated serovars were Enteritidis (64.2%), *Salmonella enterica* subsp. *enterica* rough strains (18%) and Typhimurium (5.3%) (64). In fattening pigs, 12.7% *Salmonella* positive animals were observed. Serovar Typhimurium (55.2%) was the most frequently detected serovar, followed by salmonellae belonging to serogroup B (19.9%) and serovar Derby (8.9%) (66). Among cattle herds 10.9% were tested positive for *Salmonella* with a high prevalence of serovars Dublin (38%), Typhimurium (37.3%) and Enteritidis (8.5%) (99).

It was shown that in Europe and the United States *S. enterica* serovar Typhimurium is the most frequently isolated serovar from pigs and pig products (75). Most of these strains belong to the unique phage type DT104 emerging in pigs worldwide since the early 1990s (126). Phage type DT104 is associated with enhanced virulence and multi-drug resistance (30) and usually harbours a penta-resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline (ACSSuT) (47,236). Human outbreaks caused by DT104 occur periodically.

S. enterica serovar Enteritidis is commonly isolated worldwide from poultry, especially chicken (119). The cause of infection is often asymptomatic (234). Pandemic spreading of serovar Enteritidis might have started in the mid-1980s (254). Hen house conditions involving birds and eggs as well as the human host may have contributed to the spread (87). In the 1990s the phage types PT1 and PT4 of serovar Enteritidis were most predominant within humans and poultry (146). However, between 1998 and 2003 a dramatic shift in the proportion of phage types affecting humans in Western Europe was recognised (72). Mainly PT8, PT14b and PT21 phage types replaced PT4.

1.2.1 Epidemiology of serovars commonly found in broilers and biological properties

In Western Europe including Germany *S. enterica* serovars such as 4,12:d:- or Paratyphi B dT+ have been isolated within recent years frequently from poultry, especially broilers (65,242). A European baseline survey on the prevalence of *Salmonella* in commercial broiler flocks of *Gallus gallus* in 2005 and 2006 showed that at EU-level approximately 23.7% of broiler flocks were *Salmonella* positive (65). The five predominantly isolated *Salmonella* serovars in Europe were serovars Enteritidis (33.8%), Infantis (22.0%), Mbandaka (8.1%), Hadar (3.7%) and Typhimurium (3.0%). However, the *Salmonella* prevalence and serovar distribution varied widely among the EU-Member States. In Germany, the flock prevalence of *Salmonella* was 17.5% among 378 broiler flocks investigated. The predominating serovar was the monophasic serovar 4,12:d:- with a prevalence of 23.6%. This serovar was isolated in Denmark and the United Kingdom as well with a prevalence of 15.2% and 2.8%, respectively. The second most frequently isolated serovar was Paratyphi B dT+ with a prevalence of 10.8%. In the Netherlands and Belgium Paratyphi B dT+ prevalence were 18.9% and 12.3%, respectively.

The German *Salmonella* National Reference Laboratory (NRL-Salmonella) has received 818 isolates of serovar 4,12:d:- serovar between 1998 and 2007 with peaks in 2001 (240

isolates) and 2004 (160 isolates) for diagnosis. Most of these isolates were found in broilers (78%), occasionally in turkey (11.6%) and feedstuff (8.4%), although rarely in pig (1.3%) and cattle (0.6%). In the last ten year period 1998-2007, the National Reference Centre for Salmonellae and Other Enterics located at the Robert-Koch Institute recognised 55 sporadic human cases of salmonellosis caused by serovar 4,12:d:- in Germany (W. Rabsch, personal communication). Similarly, in Denmark, only two human isolates in 1993 and in 2002 were isolated from humans (Eva Møller Nielsen, Statens Serum Institut, Copenhagen, Denmark, personal communication). The serovar is not yet acknowledged in the White-Kauffmann-Le Minor scheme (85). Empirically, it takes many years until a so-far undescribed antigenic formula is accepted as a new serovar. Any genotypic or specialized biological properties such as antibiotic resistance patterns of serovar 4,12:d:- have not yet been described in the literature.

Salmonella enterica subsp. *enterica* serovar Paratyphi B (1,4,[5],12:b:1,2) has been associated in the human host with two different clinical syndromes, enteric fever (systemic) and self-limiting gastroenteritis. According to the White-Kauffmann-Le Minor scheme both types are able to distinguished by the capability to use dextrorotatory tartrate (*d*-tartrate) as a carbon source (187). The *d*-tartrate fermenting variant dT+, formerly called *S. enterica* serovar Java, normally causes gastroenteritis in animals and humans whereas the *d*-tartrate non-fermenting variant (dT-) produces typhoid-like fever exclusively in humans, although it has occasionally been isolated from dairy cattle.

Since the end of the 1990s serovar Paratyphi B dT+ has become increasingly prominent. A number of human outbreaks have been associated with this variant in France (54), Canada (82,228), Australia (8) and several European countries (53). A particular multi-drug resistant serovar Paratyphi B dT+ clone is regularly isolated with high prevalence in poultry and poultry products in Germany and in the Netherlands (164). This clone occasionally affects humans in the Netherlands (243) and spread to other countries by the export of contaminated poultry meat from the Netherlands (32).

Miko *et al.* (165) showed that the predominant serovar Paratyphi B dT+ type associated with poultry in Western Europe possesses a chromosomally located Tn*7*-like class 2 integron carrying a *dfrA1-sat1-aadA1* gene cassette encoding resistance against trimethoprim, streptomycin, and spectinomycin. Additionally resistances to sulphonamides, nalidixic acid, and ampicillin occurred in a significant proportion of strains. Two specific pulsed-field gel electrophoresis profiles, namely X7 and X8, were associated with this type.

Another clonal line of serovar Paratyphi B dT+ has become rather widely distributed worldwide. It possesses the *Salmonella* Genomic Island 1 (SGI-1) first described in serovar Typhimurium DT104 (31) (further properties of SGI-1 see section 1.5.7). This clone was initially isolated from a tropical fish in Singapore in 1997 (162) and subsequently from humans in Canada (169), Great Britain (237) and Australia (133). These strains are generally resistant to ampicillin, chloramphenicol, streptomycin, spectinomycin, sulphonamides and tetracyclines. A characterisation study of 47 clinical isolates of multi-drug resistant serovar Paratyphi B dT+ collected between 2000 and 2003 in France revealed that 83% of the isolates possessed the SGI-1 or a variant of this cluster (257). The clonal line has not yet been found in poultry and is so far only associated with tropical fish aquaria (133) and cattle (68).

Until recently, little data describing virulence determinants of serovar Paratyphi B dT+ beyond the SGI-1 characterisation has been published (257). Prager *et al.* (192) developed a scheme to distinguish between and within serovar Paratyphi B dT+ and serovar Paratyphi B dT- isolates based on the presence or absence of the virulence genes *sopB*, *sopD*, *sopE1*, *avrA* and *sptP* as well as other molecular properties. Four enteric pathovars and two systemic pathovar variants could be defined. The genomic content of two serovar Paratyphi B dT+ isolates has been determined by microarray analysis in comparison to other subspecies I serovars but the source of strains was not indicated (188). The gene profile of one of the isolates was highly similar to one serovar Dublin isolate and might indicate a close evolutionary relationship of these serovars.

1.3 Disease and pathogenesis

Salmonella is able to cause a number of different disease syndromes, e.g. gastroenteritis, bacteraemia, enteric fever and focal infections (74). The most common disease of salmonellosis is gastroenteritis. Symptoms of gastroenteritis caused by *Salmonella* include vomiting, fever, diarrhoea, and abdominal cramps 12 to 72 hours after ingestion of the bacterium. The symptoms are normally self-limiting, resolving completely within a week. Ubiquitous *S. enterica* serovars, such as Typhimurium and Enteritidis, usually induce gastroenteritis in a broad range of unrelated host species and humans (28,171). However, the severity of infections can vary by several factors like, serovar, strain, infection dose, properties of the contaminated food (e.g. fat content) and the host's state of health. Strains of the same serovar may also differ within their virulence. Children, old or immune compromised people and pregnant women are more susceptible to developing salmonellosis than healthy adults (55,240). *Salmonella* is able to survive and multiply inside host cells. The

route of salmonellae during infection can be manifold and, however, the pathogenicity of *Salmonella* is still not completely understood. The anterior step in establishing infection is adhesion of bacteria to host epithelial cells. Specialized complementary molecules are required for ligand-receptor interaction between bacterial surfaces and host tissues (116). *Salmonella* as well as many other of the *Enterobacteriaceae* generates type 1 fimbriae, the most widely used type of fimbrial mechanism (58). In general type 1 fimbriae are expressed by a family of rod-shaped organelles which are 7 nm in diameter and 0.2–2.0 μm long (46). Type 1 fimbriae expressed by serovar Typhimurium were shown to cause persistent infection in swine (4). Moreover, type 1 fimbrials modulate bacterial gut tropism as well (231). Over 80% of *Salmonella enterica* isolates encode and express this type of fimbriae suggesting that type 1 fimbriae plays an important role in some stages of *Salmonella* invasion and life cycle (43).

Salmonellae invade the intestinal mucosa and multiply in the gut-associated lymphoid tissues (GALT). From the infected tissues the pathogens spread to the regional lymph nodes, where macrophages form a first effective barrier to prevent further spread. In cases when macrophages are unable to avoid spreading, *Salmonella* enter the blood stream and systemic diseases may occur. During systemic infections bacteria spread from the GALT via the efferent lymphatic system and the thoracic duct into the vena cava. From the bloodstream salmonellae spread throughout the body. The bacteria multiply in the spleen and liver and are released in large numbers into the blood stream infecting other organs. Symptoms include high fever (typhoid fever) and anorexia caused by lipopolysaccharide (LPS)-mediated release of cytokines. In rare occasions convulsions and delirium may appear. The use of antibiotics is only needed in severe cases of infection. A normal course of infection is cured without antibiotics. Although rare, non-typhoidal salmonellae can cause systemic disease, typically when the host's defence is compromised. Specific non-typhoidal serovars appear to be associated with rather high ratios of invasiveness compared to other *S. enterica* serovars, for example, Dublin, Heidelberg, Brandenburg and Virchow (108).

1.4 Molecular typing and characterisation methods

Sub-typing food-borne pathogens is an approach often applied to facilitate the epidemiological investigation of outbreaks of gastrointestinal disease and to identify the source of entry into the food chain. Phenotypic methods have a long record of use to characterise and trace *Salmonella* isolates. The most common way to differentiate *Salmonella* isolates is by serotyping and phage typing (see section 1.1). Another common phenotypic

method is the generation of antimicrobial resistance profiles. Many *Salmonella* isolates show resistances to multiple antibiotics belonging to the same serovar or phage type e.g. serovar Typhimurium phage type DT104 (47). In combination with serotyping data, geographical locations of the isolation and occurrence of resistance to certain antimicrobials the resistance profile provides a tool to subtype isolates.

In the last two decades, multiple molecular-based tools have been developed to type bacteria genotypically in order to determine their relatedness. Generally, molecular typing techniques are based on restriction enzyme digestion-based methods, polymerase chain reaction (PCR) amplification-based methods and sequencing-based methods (74). Here, we explain only the most relevant techniques which have been used in this thesis for the differentiation of *Salmonella* isolates.

1.4.1 Molecular characrerisation of genectic setting

Over the last ten years ago the DNA microarray technology has developed. DNA microarray analysis can serve as a promising alternative to PCR. The miniaturisation and recently commercialization of this technique in combination with advanced scanning devices enable the screening for a large set of targets simultaneously. Although microarrays have been used mainly for gene expression studies in transcriptomics, they are becoming increasingly popular in diagnostic microbiology, as well as comparative genomic hybridisations. In general, various different platforms have been developed (29). The solid supports can either be glass microscope slides, silicon chips, nylon membranes, three dimensional chips or even electrochips. Microarrays can be spotted, printed or the probes can be synthesized directly onto the support (for the example Affymetrix), and spots can either be DNA, cDNA or oligonucleotides (39). The basic principle of microarray analysis is the same as in Southern blotting (probe hybridisation), namely that complementary nucleotide sequences in DNA (or RNA) will match up. A DNA microarray consists of an orderly arrangement of spotted probes on a solid support. Owing to the use of highly accurate robotic spotters, the density of spots on an array can be very high. This high density of probes makes it possible to analyse RNA/cDNA expression of a large set of genes or to screen for the presence of a large set of targets (genes, mutations) simultaneously.

New applications are now being applied for screening large numbers of targets associated with antibiotic resistance or pathogenicity. Whole genome DNA microarrays have successfully been applied in comparative genomic hybridisations (CGH) for *Salmonella*

(21,188,197). However, whole genome arrays reflect only one genome of one strain. Because of the many serovar or strain genome variations described for *Salmonella*, thematic arrays have been developed, such as arrays specially targeting genes involved in resistance profiles (15,151,241), phage types (182) or serovars (255,266). A condensed selection of 109 *Salmonella* genetic markers comprising the detection of flagellar and somatic antigen encoding genes, important virulence genes, phage-associated genes and antibiotic resistance determinants have been used to show the usefulness of DNA microarrays for the discriminative characterisation of *Salmonella* serovars (188). Porwollik *et al*. (188) and Arrach *et al*. (11) proposed the notation "genovar" distinguishing these strain groups sharing distinct gene content, from serovars.

1.5 Molecular characteristics of the *Salmonella* genome

Salmonella is comprised of a large circular chromosome consisting of approx. 4.8 mega bases (Mb). Extra-chromosomal DNA can be present in form of plasmids having various sizes. Several *Salmonella* whole genome sequencing projects are ongoing worldwide. The Sanger Centre sequenced a number of *Salmonella* serovars e.g. *S. enterica* serovars Typhi (180), Typhimurium (49,157), Enteritidis and Gallinarum (233), other projects sequenced *S. enterica* serovar Choleraesuis (95). Until now 26 whole genomic *Salmonella* sequences are available online (22).

The plasticity of the genome describes the dynamic character of the genomic organisation and enables the bacteria to adopt selection advantages. The structure of bacterial genomes consists of an endo-genome, the core of genes and an individual set of accessory elements the exo-genome (170). In many cases insertion/deletion differences can be detected in isolates of the same serovar. These and other DNA elements such as transposons, retrons, prophages, Pathogenicity Islands, plasmids are putatively acquired or lost by horizontal gene transfer. Horizontal gene transfer is believed to be a major contributor to *Salmonella* evolution (189).

1.5.1 Salmonella *Pathogenicity Islands and Islets*

Many genes with known contribution to pathogenicity are assembled in large, unstable genomic regions of *Salmonella*. These Pathogenicity Islands (PAIs) are defined through possession of several characteristics that set them apart from other Genomic Islands (94). PAIs carry many virulence genes which are absent from related but non or lesser pathogenic species. PAIs have been acquired by horizontal gene transfer (96,189). Therefore, they are

detectable through a different G+C-content and codon usage of the island relative to the genome backbone. There is a tendency for PAIs that they inserted adjacent to tRNA genes and/or insertion sequences (IS) elements. However, the transfer of genetic encoded information is not limited to virulence. Other genetic information e.g. metabolism genes can be transferred too. Loci associated with metabolism are called genomic or fitness islands (3,16,31,134). A number of PAIs have been identified in *Salmonella* by sequence comparisons (102,156,180,247). The most prominent *Salmonella* Pathogenicity Islands are SPI-1 to SPI-5 and SPI-7. They are distinguished by numeration based on chronological order of their discovery (Figure 1) (102). They will be described here in more detail. Altogether, based on whole genome comparisons, currently, seventeen SPIs have been defined (247).

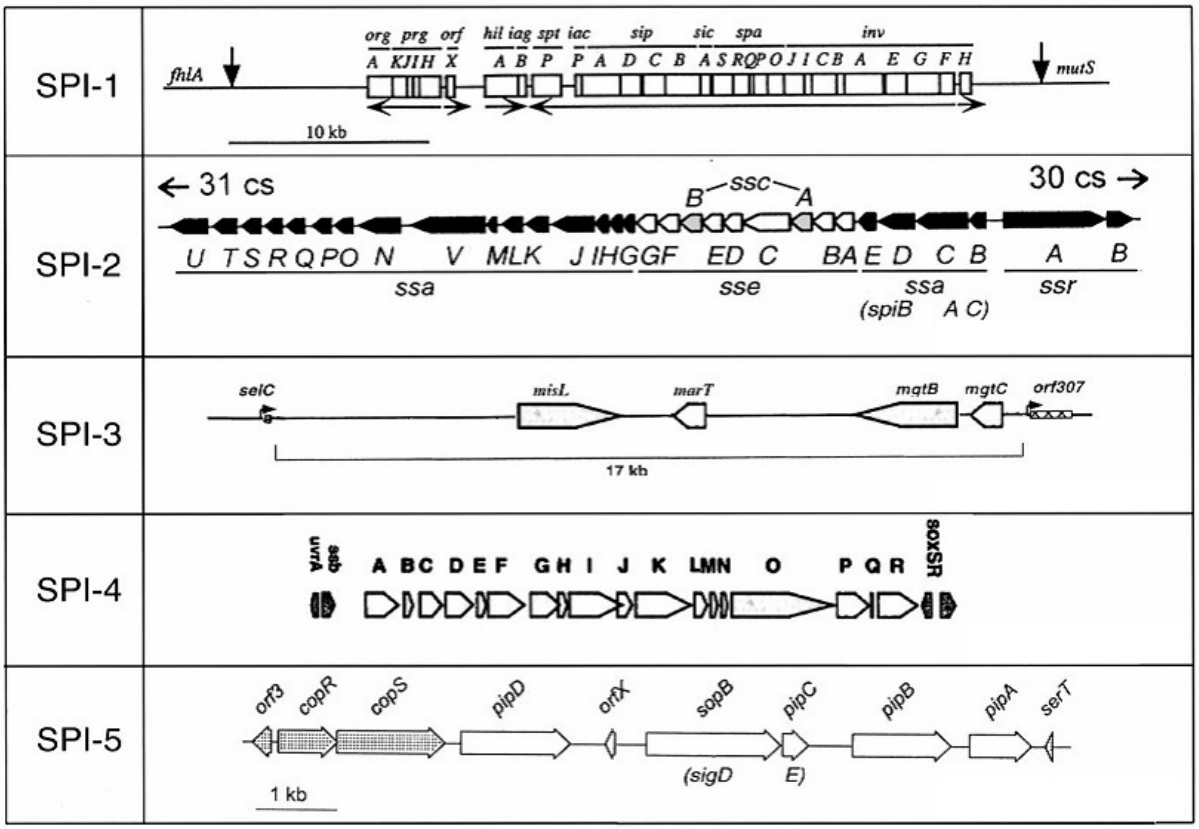


Figure 1: Schematic presentation of *Salmonella* Pathogenicity Islands 1 to 5 (modified from Marcus *et al.* (154))

Salmonella Pathogenicity Island 1 (SPI-1) has a size of 40 kb and encodes a type III secretion system (T3SS), including translocated effectors, chaperons, regulation components and an iron uptake system (Sit ABCD). The T3SS was identified in many different species like *Salmonella*, *E. coli*, *Yersinia*, *Shigella* and *Pseudomonas* enabling bacteria to translocate effector proteins into eukaryotic host cells inducing virulence reaction effects (48). SPI-1 enables invading non-phagocytic host cells and plays a role during *Salmonella* induced inflammable immune responses (115,212). The injection of a set of effector proteins in the host cell leads to a rearrangement of the cytoskeleton inducing the uptake of the bacterium by

macropinocytosis (123). After internalization of *Salmonella* containing vesicles (SCV) into eukaryotic host cells, proteins are essential for growing and maturation (106). Within this phase of invasion important effectors, like SopE, SopE2 and SopB affect small GTPases of Rho-family changing signal transduction. SopE and SopE2 operates as G-nucleotide exchanging factors on CDC42 and Rac1 (80). Other effectors like SptP mediates restitution of by SipA and SipC modified cytoskeleton through GTPase activity. SipA and SipC directly induce polymerisation and assembly of actin filaments (158). The expression of all SPI-1 encoded genes is controlled by a local system and modulated by a global regulatory system. The central, expression stimulating factor is formed by *hilA* a member of the OmpR/ToxR-regulators (2). Expression of SPI-1 encoded genes can differ because of environmental influences like pH-value, oxygen or antimicrobial peptides (12). These influences are controlled by a set of two-component regulatory systems BarA/SirA (2,230), OmpR/EnvZ (145) and PhoP/PhoQ (181) (see Fig. 3). These regulators stimulate or repress expression of SPI-1 encoded genes by activating expression of *hilE* or *hilD*, two important mediators of SPI-1 and SPI-2.

A second T3SS expressed within the intracellular phase is encoded by SPI-2. Its genes are necessary for survival and persistence within *Salmonella* containing vacuoles (SCV) and modulate vesicle transport within the host cell accommodating sufficient supply in the vesicle. Moreover, *Salmonella* growth inside the vesicles is protected from bactericidal substances (38,246). Three effectors known to have importance are encoded by SPI-2: *spiC*, *sseF* and *sseG*. SpiC blocks fusion of lysosomes with SCV (239). SseF and SseG have effects on exocytosolic transport processes (127). Like SPI-1, SPI-2 harbours a set of genes encoding metabolic proteins (104). As shown by Hensel *et al*. (105) the adjacent encoded tetrathionate reductase (*ttr*) has no important role in virulence. Similar to SPI-1, SPI-2 is regulated by a local system and modulated by a global regulatory system. The SsrA/SsrB encoded two-component regulatory system is essential for the expression of SPI-2 regulon within intracellular bacteria (44). Two-component global regulators with effects on both SPI-1 and SPI-2 gene expression are OmpR/EnvZ (81) and PhoP/PhoQ (25) as well as SlyA (173) and Fis (139).

SPI-3 is inserted at the *sel*C tRNA locus of *S. enterica* serovar Typhimurium. One of the best characterised virulence factors is the *mgt*C*B* operon. It was shown that this operon supports survival in macrophages, virulence in mice and growth under absence of Mg^{2+} (26). It encodes for a high affinity Mg-uptake system with adapted expression to nutrient-poor conditions existing within SCV's (224). Another important encoded protein, MisL, is a

classical auto-transported protein with significant similarities to AIDA-1, an adhesion protein of enteropathogenic *E. coli*. Moreover, *misL* encodes an adhesion protein enabling persistence within mice (118). The distribution of SPI-3 sequences varies among the salmonellae. The right end of this 17-kb island is present in all species whereas a four-gene cluster in the central region is only found within some subspecies (27).

The 24 kb SPI-4 contains six open reading frames, namely *siiA* to *siiF* (150). Wong *et al.* (260) first described it in 1998. A type one secretion system (T1SS) encoded by *siiC*, *siiD*, and *siiF* secretes SiiE a 595-kDa protein. A decreased SiiE concentration within SPI-1 mutant strains suggests that SPI-1 and SPI-4 may have common regulatory inputs. In addition, SiiE appears to be required for long-term survival (129). It was shown that mutations in *hilA*, *hilC*, or *hilD* reduce expression of SiiE, whereas mutations in *hilE* or *phoP* enhance SiiE expression (150). In absence of SPI-1 individual over expression of HilA, HilC, or HilD does not activate SiiE expression, suggesting a simultaneous action of these transcriptional regulators or action in combination with additional SPI-l-encoded regulatory loci activating SPI-4 (18).

SPI-5 is a 15 kb island (261). The island is absent in *S. bongori* but within *S. enterica* subspecies widely distributed. The SPI-5 located genes *sopB*, *pipD* and *pipB* encodes proteins that can influence secretory responses during enteritis in the bovine ligated ileal loop model (261). SopB mediates cytoskeleton rearrangements and bacterial entry by changing signal transduction through production of second messengers which affects small GTPases of Rho-family (268). A defective SopB mutant failed to stimulate cytoskeleton rearrangements.

SPI-7 is a locus that has been found only in serovars Typhi, Paratyphi C and Dublin (185). The locus has a size of 133 kb and is inserted adjacent to tRNA *pheU* (97). Several virulence factors are located on SPI-7. It encodes the Vi-antigen, a capsular exo-polysaccharide, the *sopE* phage encoding effector protein SopE and a putative type IVB pilus encoded by *pil* gene cluster (185).

Many other smaller regions have been identified in serovar Typhimurium which have been shown to encode genes contributing to virulence but are not necessarily associated with any transferable elements. These regions are called Pathogenicity Islets. For example, a small islet of STM2513-2518 encodes *shd* and *ratB* involved in fecal shedding and colonization of the murine cecum (122). Another islet is STM0854-STM0859 and STM3117 and STM3120 which are upregulated during macrophage infection (63). Even single gene islets (singlets) can be involved in pathogenicity. By microarray comparisons with the serovar Typhimurium

strain LT2 genome approximately 50 such singlets have been found, often with unknown function (190). Toxins which are released in the environment are not known for *Salmonella*.

1.5.2 Prophages

Bacteriophages are viruses which use bacteria as host cells. Many phages integrate into the host genome to form prophages. They can encode genes that alter the host phenotype in a process called lysogenic conversion. Those genes can be involved in the virulence of the bacterium. All salmonellae contain phage genomes and/or phage remnants in their chromosome. Four serovar Typhimurium LT2 four functional prophages have been found, namely Fels-1, Fels-2, Gifsy-1 and Gifsy-2 (71,226). The serovar Typhi genome of strain CT18 contains seven prophage genomes (180). The vast majority belongs to the P2-like family. Three other fully functional lysogenic phages are termed Gifsy-3, sopEϕ and P22 found in other *Salmonella* strains (71,167). Table 2 shows important virulence genes found on these prophages.

Table 2: Important prophage virulence genes (modified from Porwollik *et al*. (189))

Phage	Phage type	Important	Characteristics of gene product	Reference
Fels-1	Lambdoid	*sodCIII*	Superoxide dismutase	(71)
		nanH	Neuraminidase	(71)
Fels-2	P2			(33)
Gifsy-1	Lambdoid	*gipA*	Critical for survival in Peyers patches	(226)
Gifsy-2	Lambdoid	*sodC1*	Superoxide dismutase, high catalytic	(70)
		sseI	Type III translocated	(163)
		gtgA	Virulence gene, ess. for full virulence	(107)
Gifsy-3	Lambdoid	*sspH1*	Type III effector protein	(71)
		pagJ	*phoPQ*-activated gene	(71)
sopEΦ	P2	*sopE1*	Type III effector protein	(166)
P22	P22	*gtrA*	O-antigen conversion, flippase	(245)
		gtrB	O-antigen con., glycosyl transferase	(245)

1.5.3 Salmonella *virulence plasmid*

Other virulence factors found in *Salmonella* can be encoded on plasmids. In several *Salmonella enterica* subsp. *enterica* serovars, especially in serovars Typhimurium, Enteritidis, Choleraesuis, Dublin Gallinarum, and Typhi plasmids have been identified on which virulence genes have been identified that are important for systemic infections (92,251). These so called *Salmonella* virulence plasmids can vary in size between 30 and 100 kb for different serovars indicating a mosaic plasmid composition (262). The factors encoded are associated with enhanced virulence such as the *spv* cluster which is essential for infection in laboratory rodents. However, the role of virulence plasmids in gastroenteritis and invasive disease in humans is still unclear. Some reports suggest that a highly conserved horizontal acquired operon *spvABCD* (*Salmonella* plasmid virulence) promotes dissemination of serovar Typhimurium from the gut (69). Plasmid carrying strains show an increase of the growth rate of bacteria in the liver and spleen (196). Its expression is controlled by *spvR* which is itself regulated by growth conditions like the disposability of nutrients (259). The plasmid encoded fimbrial operon *pefABCD* supports colonisation by adhesion to gut epithelial cells. Infection studies within mice showed a decreased infectivity by the simultaneously inactivation of four fimbrial loci including *pef* (244). Resistance to complement killing is conveyed by the plasmid encoded Rck. Heffernan *et al*. (100) showed that Rck impedes the final polymerization of C5b-9 membrane attack complex.

1.5.4 Fimbrial clusters

The first step of infection is carried out by attachment (adhesion) of *Salmonella* to host cells (see 1.3). During adhesion, bacterial cells bind to surfaces through hair-like fimbriae. An abundance of these operons is encoded by *Salmonella*. Serovar Typhimurium harbours 11 different chromosomally encoded fimbrial loci and additionally one plasmid encoded (*pef*) locus, whereas serovar Enteritidis (233) encodes 13 fimbrial clusters, 10 of them are shared by serovar Typhimurium strain LT2.

Different fimbrial types include: mannoside-binding type 1 fimbriae (encoded by *fim* cluster), plasmid encoded fimbriae (*pef*), long polar fimbriae (*lpf*), thin aggregative fimbriae also called curli (*agf/csg*), bovine colonisation factor (*bcf*), *Salmonella* Typhimurium fimbriae (*stf*), *Salmonella enterica* SEF14 fimbriae (*sef*) and *Salmonella* atypical fimbriae (*saf*) (17-20). *Salmonella* adheres to different cells including intestinal cells, respiratory cells, erythrocytes, leukocytes, protozoa, yeast, fungal hyphae, and plant root hairs (14,125).

Adhesion of type 1 fimbriae forms the first step in virulence as shown by several studies (4,132). It was shown that type 1 expressing fimbriae are more virulent than non-Fim expressing strains (59,244).

1.5.5 Antimicrobial resistance and resistance determinants

Because of the extensive use of antimicrobial agents in animal and human therapy resistance became a public health concern. During the last decades antimicrobial resistance in *Salmonella* developed tremendously. The development of resistance in *Salmonella* toward antimicrobial agents is attributable to one of multiple mechanisms, including production of enzymes that inactivate antimicrobial agents, through degradation or structural modification, reduction of bacterial cell permeability to antibiotics, activation of efflux pumps and modification of cellular targets for drugs (217). Many strains, especially serovar Typhimurium, are multidrug resistant to five or more antimicrobial agents (235) originating from the chromosomally located *Salmonella* Genomic Island 1 (SGI-1) (31).

ß-lactamases are a diverse group of enzymes, some with affinities for the structures of a limited number of antimicrobial agents, whereas others are extended-spectrum ß-lactamases (ESBLs) degrading a wide range of antibiotics. Several families are known, namely AmpC (encoded by bla_{CMY}), TEM (encoded by bla_{TEM}), SHV (encoded by bla_{SHV}), OXA encoded by bla_{OXA}), and CTX-M (encoded by bla_{CTX}) (177).

Enzymes inactivating the antimicrobial agents by modifying their structure occur in amino glycoside resistance. These enzymes include amino-glycoside phosphotransferase encoded by *aphA* (responsible for kanamycin resistance), amino-glycoside acetyltransferase encoded by *aacC* (responsible for gentamicin resistance) and amino glycoside-adenyltransferase encoded by *aadA* and *aadB* (responsible for streptomycin and gentamicin resistance).

Resistance to fluoroquinolones is mainly mediated by point mutations within the QRDR region of the *gyrA* and *gyrB* genes (encoding DNA gyrase) and/or *parC* and *parE* (DNA topoisomerase IV). Recently, several plasmid-mediated genes (*qnr*) encoding quinolone resistance have been reported in *Salmonella* (201). The *qnr* gene family shows high diversity. There are at least 6 *qnrA*, 20 *qnrB*, and 3 *qnrS* alleles known (http://lahey.org/qnrStudies). The Qnr proteins are capable of protecting DNA gyrase from quinolones. AAC(6')-Ib-cr, a variant amino glycoside acetyltransferase capable of modifying ciprofloxacin and reducing its activity, seems to have emerged more recently, but might be even more prevalent than the Qnr protein (201).

Much of the resistance to tetracycline and chloramphenicol is associated with the acquisition and expression by efflux pumps that remove toxic of the drug from the bacterial cells. In *Salmonella* tetracycline efflux pumps are encoded by the *tet* genes (78), whereas chloramphenicol efflux pumps are encoded by *floR* or *cml* (34). The *cat* gene encoding a chloramphenicol acetyltransferase confers also resistance against chloramphenicol.

The drugs trimethoprim and the sulfonamides inhibit different enzymes in the folic acid biosynthetic pathway in bacterial cells. Resistance to sulfonamides in *Salmonella* is often caused by the presence of either *sul1*, *sul2* or *sul3* (10) which encode altered dihydropteroate synthetase enzymes that have reduced affinity for sulfonamides, but function in the folic acid biosynthesis (113). Similarly, trimethoprim resistance is conferred by *dhfr* genes encoding an altered dihydrofolate reductase with reduced affinity for the antimicrobial agent.

1.5.6 Elements associated with DNA-mobility

Several different types of mobile elements have been described playing an important role in the acquisition, maintenance and spread of antimicrobial resistance genes (149,208,216). Plasmids, transposons, and integrons represent most important genetic elements. These mobile genetic elements can spread horizontally among bacteria of same serovars, subspecies, or species, but also among those of different species or even different genera (232).

In contrast to plasmid harbouring replication systems (52) transposons do not possess replication genes and integrate into chromosomal or plasmid DNA for replication. Long terminal direct repeats or inverted repeats are characteristic for composite transposons deriving from structures developed from insertion sequences (IS). The first transposable elements identified were IS-elements (213). IS-elements consist of terminal inverted repeats of variable length and a transposase gene (148). Composite transposons, still function as independent elements, but lose this ability after fusion processes (172). Examples of composite transposons are Tn*5* (172) encoding resistance genes against amino-glycosides and Tn*7* (144) carrying genes encoding for resistance against trimethoprim and amino-glycosides. Two examples for complex transposons spread in *Salmonella* of this type of transposons are the resistance Tn*3* and Tn*21* transposons (86,202).

Several classes of integrons have been described based on sequence differences in integrase genes (*intI*) (176,205). The most prominent and widely spread integron among *Salmonella enterica* and also other gram-negative bacteria belongs to the class 1 integron (83,90,141,195,258,267). Class 1 integrons are largely associated with Tn*21*-related

transposons (202). Tn*21* like transposons are mostly encoded by large self-transferable plasmids primordial isolated from *Shigella flexneri* (138). Class 2 integrons, share structures with the class 1 integrons within its 5'-CS region but encodes a distinct integrase (*intI2*) including an internal stop codon occurring within the Tn*7*-transposons family. Tn*7* can encode a *dfrA1*-*sat1*(Tn*7*)-*aadA1* gene cassette procuring resistance to trimethoprim, streptomycin and spectinomycin (227). However, until now, class 2 integrons are not common, they have been found in *S. enterica* subspecies I serovars Paratyphi B dT+, Enteritidis, Virchow and Typhimurium (141).

1.5.7 Salmonella *Genomic Island 1 (SGI-1)*

SGI-1 is a chromosomal gene cluster of variable length (up to 43 kb) (31) (Fig. 2). It was originally found in a Canadian *S. enterica* serovar Typhimurium phage type DT104 isolate but was recently also detected in other phage types of serovar Typhimurium (37), and other epidemic *Salmonella enterica* serovars e.g. Agona, Albany, Newport and Paratyphi B dT+ (30,134). SGI-1 is located between the *thdf* and *int2* gene and flanked by direct repeats. The typical multidrug-resistance phenotype ACSSuT has been found on a 14 kb region belonging to SGI-1. It contains two class I integrons, each with a single gene cassette harbouring *aadA2* (streptomycin/spectinomycin resistance), and bla_{PSE-1} (ampicillin resistance), respectively, as well as additionally *floR* (chloramphenicol/florfenicol resistance), *tet*(G) (including *tetR* and *tetA*) (tetracycline resistance) and *sul1* (sulfonamides resistance). Variants on these resistant pheno/genotypes have been described (30,40,57). The remaining part of SGI-1 encodes genes showing significant homology to plasmid-related and phage-related genes.

SGI-1 has the ability to transfer horizontally (56). Chromosomal integration occurred through site-specific recombination between an 18 bp sequence located within SGI-1 and the chromosome on the 3' side of the *thdF* gene.

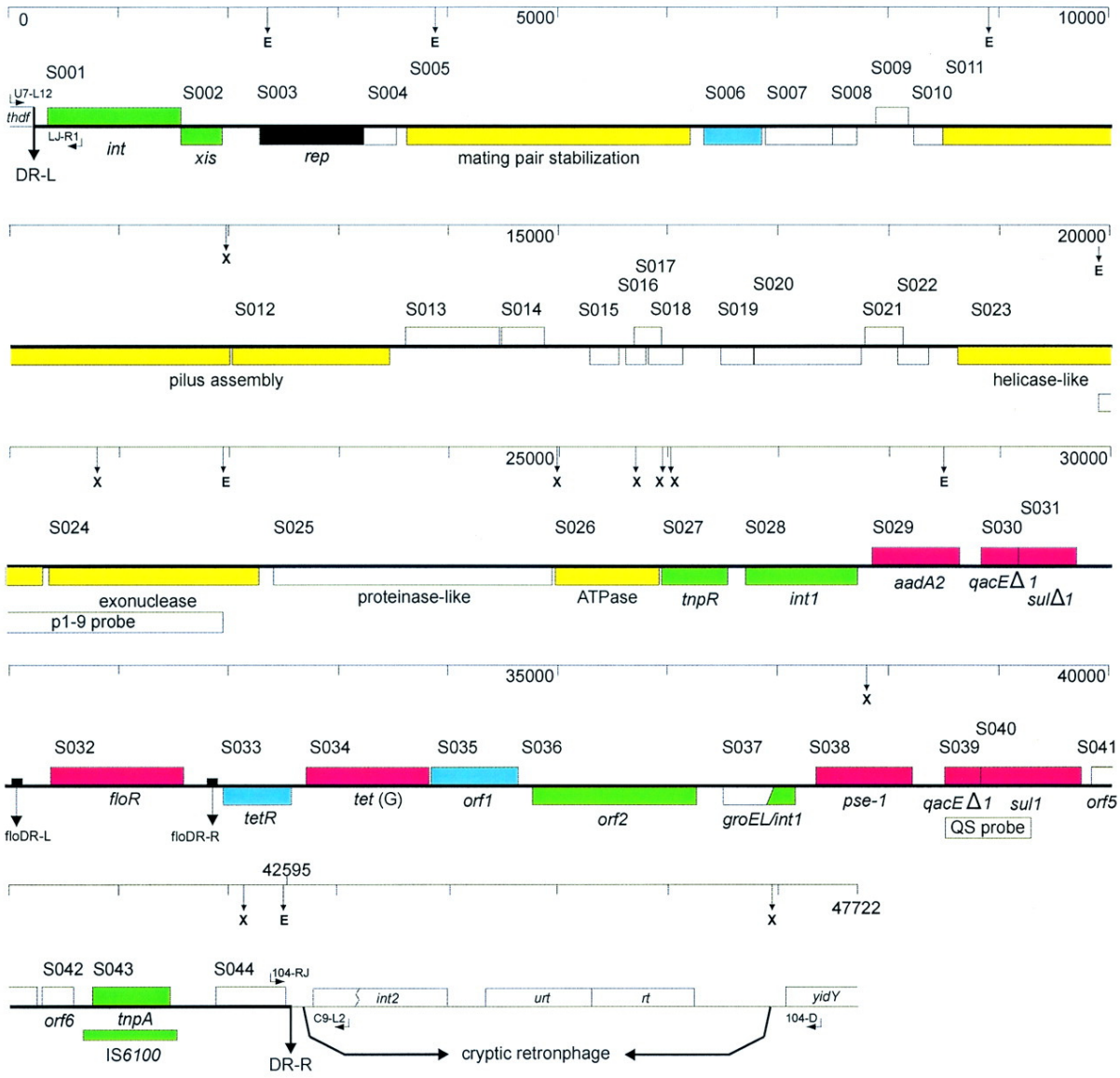


Figure 2: Linear representation of the complete SGI-1 and flanking regions. Upper rectangles indicate ORFs transcribed from right to left, and lower rectangles are transcribed left to right. GenBank entries of ORFs were assigned unique identifiers in the form SXXX. Colour coding indicates ORFs with similar function as follows: gray, DNA recombination; black, DNA replication; light gray, conjugal transfer; gray, regulatory; dark gray, drug resistance; white, not known or other functions. Copied from Boyd *et al*. (31).

1.5.8 Genes involved in serovar identification according to White-Kauffmann-Le Minor scheme

Genes specific for synthesis of the somatic O-antigens are generally located in the *rfb*-cluster encoding enzymes for the synthesis of lipopolysaccharide-core and -side chain sequences, for their transport by O-antigen transporters (*wzx*) (142) and for their assembly into oligosaccharide units by O-antigen polymerases (*wzy*) (73). Several *rfb*-clusters have been sequenced (73,198,252). Extensive genetic variation in *rfb*-clusters of *Salmonella* induces O-antigen variance (73,265). This diversity is mainly based on differences in

composition of sugars, arrangement of sugars in the O-core unit, specific linkages between O-units, addition of branch sugars and modified side chains (198). Some further antigen polymerases (*rcf*, *oaf*, *gtrAB*) are encoded at other regions of the genome (189,221). The structural first phase flagellin antigen (H1) is encoded by the *fliC* gene and the structural second phase flagellin antigen (H2) is encoded by the *fljB* gene (270). Both genes are expressed co-ordinately by a phase-variation mechanism (219). The alternated expression is controlled by inversion of an 800-base-pair sequence (*hin* locus) which is located adjacent to the *fljAB* operon (269). Both structural genes have highly variable regions embedded in rather conserved framework. The White-Kauffmann-Le Minor scheme describes 54 variations of the H1- and 44 of the H2-antigen (85). Several sequences of *fliC* and *fljB* have been published (114,117,223).

1.6 Aim of the thesis

The aim of this thesis was the development and validation of a DNA microarray for the molecular characterisation and typing of *Salmonella enterica* subspecies *enterica* isolates. The DNA microarray shall detect the presence or absence of important *Salmonella* targets presently described for *Salmonella enterica* subsp. *enterica* in terms of pathogenicity, antimicrobial resistance, mobility, metabolism and serotyping. The number of probes should not exceed 300 so as to handle data generation in an easy way, to allow an extensive validation of the microarray by PCR and last but not least to have a good cost/performance ratio. Proper validation was necessary because this prototype shall be able to be applied in routine diagnostics at the National Salmonella Reference Laboratory (Berlin, Germany). Such molecular data are the basis estimating the potential hazard of the *Salmonella* strains isolated from animals or humans.

The DNA microarray was applied to two *S. enterica* serovars 4,12:d:- and Paratyphi B dT+. The *S. enterica* serovar 4,12:d:- with high prevalence in broiler flocks was genotypically characterised for the first time primarily by DNA microarray and PFGE determining clonality, the pathogenic gene repertoire and resistance determinants. A particular *S. enterica* serovar Paratyphi B dT+ clone persisting in poultry and poultry products in the Netherlands, Belgium and Germany was investigated determining multi-drug resistance profiles, clonality and virulence as well as resistance gene repertoire. For both strains the hazard potential for humans is discussed.

2. Materials and Methods

2.1 Materials

2.1.1 Instruments

Table 3: Instruments

Product	Abilities	Company
AdvaTube	Slide holder tube	Advalytix, München, D
aQu split pin, K2801	Tungsten, 75 µm	Genetix, Hamshire, UK
Beaker, Erlenmeyer flask	glass, sterilized	Schott, Mainz, D
Binocular microscope		Olympus, Hamburg, D
CHEF DRIII PFGE	Variable Angle current System	BioRad Laboratories GmbH, München, D
Concentrator	Model 5301	Eppendorf, Hamburg, D
Desiccator	polycarbonate	Neolab, Heidelberg, D
DNA Electrophoresis Gel Box	horizontal	BioRad Laboratories GmbH, München, D
EagleEye-II	gel documentation system	Stratagene, La Jolla, CA, USA
GenePix 4000B	two colour laser scanner	Axon Instruments, CA, USA
Glass chamber with slide rack	Schiefferdecker type	Duran, Mainz, D
Glass flasks 0.2 l; 0.5 l; 1.0 l	glass, sterilized	Schott, Mainz, D
Hood HeraSafe		Heraeus, Hanau, D
Magnetic stir bar	different sizes	Neolab, Heidelberg, D
Microarrayer Qarray mini		Genetix, Heidelberg, D
Micro centrifuge 5402	chilled	Eppendorf, Hamburg, D
Mixer Heidolph MR 3001	heatable, continuously adjustable	Heidolph Instruments, Schwabach, D
Multichannel pipette	12*100 µL	Eppendorf, Hamburg, D
Multipette	25 µL – 5 mL container	Eppendorf, Hamburg, D
Pipettes Eppendorf Research	2.5 µL, 10 µL, 200 µL, 1000 µL	Eppendorf, Hamburg, D
Pipetboy pipetus-akku		Hirschmann, Eberstadt, D
PowerPac Basic	Power supply, 10-300 V, 4-400 mA, 75 W max.	BioRad Laboratories GmbH, München, D
sciHybchamber	Hybridisation chamber	Scienion, Berlin, D
Sensititre Autoinoculator	INO2	Trek Diagnostic systems, East Grinstead, UK
Sensititre Sensitouch System	Semi-automatic	Trek Diagnostic systems, East Grinstead, UK
Thermocycler	Model 9700	Applied Biosystems, Darmstadt, D

Thermo mixer	Model 5436	Eppendorf, Hamburg, D
Tweezers		Eppendorf, Hamburg, D
Vortex mixer Reax top	continuously adjustable	Heraeus, Hanau, D
Water bath WB7	continuously adjustable	Memmert, Schwabach, D

2.1.2 Disposables

Table 4: Disposables

Product	**Composition**	**Company**
384 well plate X7022	polypropylene	Genetix, Heidelberg, D
Amber reaction tube	1.5 mL	Eppendorf, Hamburg, D
Cell filter	70 µm	BD, Le Point de Claix, F
CodeLink	Pre-treated glass slides	GE Healthcare, München, D
DNA purification spin columns	collection tubes included	Invitrogen, Karlsruhe, D
FALCON tubes	Polystyrene, 15 mL, 50 mL	Becton Dickinson, Heidelberg, D
Filter tips	2.5 µL, 10 µL, 200 µL, 1000 µL	Eppendorf, Hamburg, D
Lifter slips	MSeries 22 mm x 26.5 mm	Erie Scientific Company, Portsmouth, NH, USA
Microtiter plate NLMV1A	coated with 17 different antimicrobials	Trek Diagnostic Systems, East Grinstead, UK
PFGE mould	Reusable	BioRad Laboratories GmbH, München, D
Parafilm		American National Can, Menasha, WI, USA
Reaction tubes	1.5 mL, 2 mL	Eppendorf, Hamburg, D

2.1.3 Bacterial strains

Twenty reference strains used for the validation of the DNA microarray were previously published (152). All 24 reference strains represented positive and negative control strains for oligonucleotide probes printed on the microarray. Their characteristics are listed in Table 5. The *E. coli* strain was used to determine the hybridisation of other *Enterobacteriaceae*. Moreover, it was selected as an positive control for the *tet*(D) gene.

For genotypic characterisation of serovar 4,12:d:-, strains were selected from the NRL-*Salmonella* collection. The strains were isolated from feed (7 strains), turkey (5 strains), broilers (24 strains) and pigs (3 strains). In addition 17 strains isolated from infected humans who suffered from salmonellosis were selected from the collection of the National Reference Centre for Salmonellae and Other Enterics (Robert-Koch Institute, branch Wernigerode, Germany) (Appendix I, Table A3). For the determination of the genetic relationship of

serovar 4,12:d:- to potentially related diphasic serovars, one *S. enterica* serovar Schwarzengrund, two *S. enterica* serovar Duisburg, and two *S. enterica* serovar Stanley strains were selected. These serovars have the same somatic (O) antigen and phase-1 flagellar (H1) antigen but express in addition phase-2 (H2) flagellar antigens (Appendix I, Table A3). Since the H2 flagellar antigen of *S. enterica* serovar Derby can be present or absent, the serovar was included in the study. Additionally, *S. enterica* serovar Livingstone, *S. enterica* serovar Infantis and *S. enterica* serovar Paratyphi B d-tartrate positive (dT+) were selected to analyse their genetic relatedness to serovar 4,12:d:- because they are frequently isolated from chicken in Germany. In humans, the highly prevalent serovars Typhimurium and Enteritidis were added for the analysis of virulence gene determinants.

S. enterica serovar Paratyphi B dT+ and *S. enterica* serovar 4,5,12:b:- strains characterised in this study were obtained from the collections of the Centre for Infectious Disease Control in Bilthoven, the Netherlands; the Veterinary Laboratories Agency in Weybridge, United Kingdom; the Veterinary and Agrochemical Research Centre in Brussels, Belgium; and the National *Salmonella* Reference Laboratory in Berlin, Germany (Appendix I, Table A4). Dutch, Belgian, and German strains were selected based on the diversity of phenotypic antimicrobial resistance profiles isolated between 2005 and 2008 from poultry, pigs, and humans. British strains were selected based on the same criteria but included isolates from environmental and other animal origins. These strains reflect a greater diversity of origin compared with the other group of strains. In addition, three *Salmonella* 4,5,12:b:- strains were isolated in Denmark from humans suffering from gastroenteritis.

Table 5: *Salmonella* reference strains used for microarray validation and their characteristics.

Reference strain no.	Serovar	Phage type	Serogroup	Antigenic formula	Phenotypic antimicrobial resistance profile [b]
LT2	Typhimurium		B	1,4,12:i:1,2	Susceptible
SUO5 [a]	Typhimurium	DT120	B	1,4,12:i:1,2	AMP-CHL-SPE-STR-SUL-TET
SUO1	Typhimurium	DT104	B	1,4,12:i:1,2	AMP-CHL-FLO-SPE-STR-SUL-TET
NRL [c] 00-419	Typhimurium	DT104	B	1,4,12:i:1,2	AMP-CHL-FLO-NAL-SPE-STR-SUL-TET-TMP-SXT
SUO6	Typhimurium	RDNC [d]	B	1,4,12:i:1,2	AMP-CHL-KAN-NEO-SPE-STR-SUL-TET-TMP-SXT
S65 (NRL 01-02571)	Typhimurium	DT104A	B	1,4,12:i:1,2	AMP-CHL-SPE-STR-SUL-TET-TMP-SXT
S40 (NRL 01-01338)	Typhimurium	DT12	B	1,4,12:i:1,2	TET
NRL 99-4068	Typhimurium	DT120	B	1,4,12:i:1,2	AMP-CHL-GEN-KAN-NEO-SPE-STR-SUL-TET-TMP-SXT
NRL 05-354	Typhimurium	DT193	B	1,4,12:i:1,2	AMP-KAN-NEO-STR-SMX-SPE-SXT-TET-TMP
SUO8	[4,5,12:i:-]	U302	B	4,5,12:i:-	AMP-CHL-GEN-SPE-STR-SUL-TET-TMP-SXT
NRL 98-3363	Schleissheim		B	4,12,27:b:-	Susceptible
NRL 01-1543	Paratyphi B (d-tartrate+)		B	1,4,12:b:1,2	AMP-AMC-NAL-SPE-STR-SUL-TMP-SXT
NRL 01-1380	Saintpaul		B	1,4,12:e,h:1,2	AMP-GEN-KAN-NAL-NEO-SPE-STR-SUL-TET
NRL 01-3160	Stanleyville		B	1,4,5,12,27:z_4:z_{23}:1,2	Susceptible
NRL 04-2860	Mbandaka		C1	6,7,14:z_{10}:e,n,z	AMP-TMP-SPE
NRL 02-102	Oranienburg		C1	6,7:m,t:-	Susceptible
NRL 99-601	Hadar		C_2-C_3	6,8:z10:e,n,x	AMP-NAL-STR-TET
NRL 01-2132	Goldcoast		C_2-C_3	6,8:r:l,w	GEN-STR-SPE-SUL-TET
NRL 00-4	Enteritidis		D1	1,9,12:g,m:-	Susceptible
RKI [e]-Ty1	Typhi	London	D1	9,12,Vi:d:-	Susceptible
NRL 98-454	Dublin		D1	1,9,12[Vi]:g,p:-	Susceptible
NRL 99-929	Anatum		E1	3,10:e,h:1,6	Susceptible
NRL 03-1949	Lindern		H	6,14,24:d:e,n,x	Susceptible
NRL EC227	*E. coli*				AMP-CHL-KAN-NEO-STR-SUL-TET-TMP-SXT

[a] SUO: *Salmonella* University of Oviedo

[b] AMP, ampicillin; AMC, amoxicillin/clavulanic acid; CHL, chloramphenicol; FLO, florfenicol; GEN, gentamicin, KAN, kanamycin; NEO, neomycin; NAL, nalidixic acid; SPE, spectinomycin; STR, streptomycin; SUL/SMX, sulfamethoxazole; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; TMP, trimethoprim
[b] NRL: National *Salmonella* Reference Laboratory
[d] RDNC reaction does not conform
[e] RKI: Robert-Koch Institute

2.1.4 Culture media

All culture media were calculated for a final volume of 1 L and if not otherwise indicated, sterilized by autoclaving for 15 min at 120°C. Antibiotics were added after autoclaving the media.

Buffered peptone water (BPW), Oxoid

Product	**Amount**
Peptone	10 g
NaCl	5 g
Na_2HPO_4	3.5 g
KH_2PO_4, pH 7.2	1.5 g
	pH 7.2 ± 0.2

Gassner culture medium, Merck KGaA

Product	**Amount**
Lactose	43 g
Peptone	14 g
Agar	13 g
NaCl	5 g
Metachrome yellow	1.25 g
Water blue	0.62 g
	pH 7.2 ± 0.2

Luria-Bertani (LB) broth and culture medium, Oxoid

Product	**Amount**
Yeast extract	5 g
Tryptone	10 g
NaCl	10 g
Agar (media)	10 g
	pH 7.0 ± 0.2

Müller-Hinton culture medium, Oxoid

Product	Amount
Meat extract	300 g
Casein hydrolysate	17.5 g
Agar	17 g
Starch	1.5 g
	pH 7.3 ± 0.2

Müller-Hinton II culture medium, Beckton Dickinson

Product	Amount
Beef extract	3 g
Casein hydrolysate	17.5 g
Agar	17 g
Starch	1.5 g
	pH 7.3 ± 0.2

Rappaport-Vassiliades culture medium, Merck KGaA

Product	Amount
Magnesium chloride hexahydrate	29 g
NaCl	8 g
Soy peptone	4.5 g
KH_2PO_4 ,pH 7,2	0.6 g
K_2HPO_4 ,pH 7,2	0.4 g
Malachite green	0.036 g
	pH 5.2 ± 0.2

Rappaport-Vassiliades was sterilized by autoclaving for 15 min at 115°C.

Xylose-lysine deoxycholate (XLD) agar, Oxoid

Product	Amount
Agar	12.5 g
Lactose	7.5 g
Sucrose	7.5 g
Sodium thiosulphate	6.8 g
NaCl	5 g
L-lysine HCl	5 g
Xylose	3.75 g
Yeast extract	3 g
Sodium deoxycholate	1 g
Ferric ammonium citrate	0.8 g
Phenol red	0.08 g
	pH 7.4 ± 0.2

XLD agar was gradually solved under shaking at 100°C.

2.1.5 Reagents and buffers

Table 6: Reagents

Product	Composition	Company
Agarose	Low melting	Invitrogen, Karlsruhe, D
dNTP Mix (A,T,G,C)	2 mM each	Roth, Karlsruhe, D
Ethidium bromide solution	10 mg/mL (w/v)	Roth, Karlsruhe, D
Exo-Klenow Fragment	5 U/ µL	GE Healthcare, München, D
Exo-Klenow Fragment	50 U/ µL	NE Biolabs, Frankfurt , D
H9812, *Salmonella* PFGE Size marker	*S. enterica* serovar Braenderup, *Xba*I restricted	in-house
Proteinase K	>600 mAU/mL	Qiagen, Hilden, D
Reaction buffer 2.5X	125 mM Tris-HCl	Invitrogen, Karlsruhe, D
RNAse A	100 mg/mL	Qiagen, Hilden, D
SeaKem Agarose	PFGE blocks	Biozym Scientific GmbH, Oldendorf, D
Sodium dodecyl sulphate		Sigma-Aldrich, Steinheim, D
Taq polymerase	5 U/ µL	Invitrogen, Karlsruhe, D
Thiourea	100 µM	Roth, Karlsruhe, D
Tween 20		Sigma-Aldrich, Steinheim, D
*Xba*I with 10X SureCut H-buffer	restriction enzyme for PFGE	Roche Diagnostics, Mannheim, D

BfR hybridisation buffer

Product	Volumes	Company
Formamide	400 µL	Sigma-Aldrich, Steinheim, D
50X Denhardt's solution	100 µL	Fluka, Basel, Switzerland
10% (w/v) SDS	100 µL	Sigma-Aldrich, Steinheim, D
20x SSC	150 µL	Sigma-Aldrich, Steinheim, D
20% (w/v) dextrane sulphate	250 µL	Sigma-Aldrich, Steinheim, D

50X Denhardt's solution (Fluka)

Product
1% (w/v) Bovine serum albumin (BSA)
1% (w/v) Ficoll 400
1% (w/v) Polyvinylpyrrolidone

Solution was filtered prior to storage through a 0.2 µM filter.

Microarray blocking solution

Product	Company
100 mM Tris HCl, pH 7.5	Sigma-Aldrich, Steinheim, D
50 mM ethanolamine, pH 9.0	Sigma-Aldrich, Steinheim, D

2X Microarray spotting (Print) buffer

Product	Company
300 mM sodium phosphate, pH 8.5	Sigma-Aldrich, Steinheim, D

Microarray wash solution I

Product	Company
150 mM NaCl, pH 7.0	Sigma-Aldrich, Steinheim, D
15 mM sodium citrate	Sigma-Aldrich, Steinheim, D
1 mM SDS	Sigma-Aldrich, Steinheim, D

Microarray wash solution II

Product	Company
30 mM NaCl, pH 7.0	Sigma-Aldrich, Steinheim, D
3 mM sodium citrate	Sigma-Aldrich, Steinheim, D

Microarray wash solution III

Product	Company
7.5 mM NaCl, pH 7.0	Sigma-Aldrich, Steinheim, D
0.75 mM sodium citrate	Sigma-Aldrich, Steinheim, D

PFGE Cell-lyses buffer

Product	Abilities	Company
5 mM Tris-HCl, pH 8.0		Sigma-Aldrich, Steinheim, D
5 mM EDTA, pH 8.0		Sigma-Aldrich, Steinheim, D
1 % (w/v) Sarcosyl		Sigma-Aldrich, Steinheim, D
Proteinase K (20 mg/mL)	Ad 25 µL per reaction	Sigma-Aldrich, Steinheim, D

PFGE Cell-suspension buffer

Product	Company
100 mM Tris-HCl, pH 8.0	Sigma-Aldrich, Steinheim, D
100 mM EDTA, pH 8.0	Sigma-Aldrich, Steinheim, D

Phosphate buffered saline (PBS)

Product	Company
137 mM NaCl	Roth, Karlsruhe, D
2.7 mM KCl	Sigma-Aldrich, Steinheim, D
8.1 mM Na_2HPO_4	Sigma-Aldrich, Steinheim, D
1.5 mM KH_2PO_4, pH 7.2	Sigma-Aldrich, Steinheim, D

Post-coupling wash solution

Product	Company
60 mM sodium citrate	Sigma-Aldrich, Steinheim, D
0.3 mM SDS	Sigma-Aldrich, Steinheim, D

20X Sodium citrate (SSC) buffer

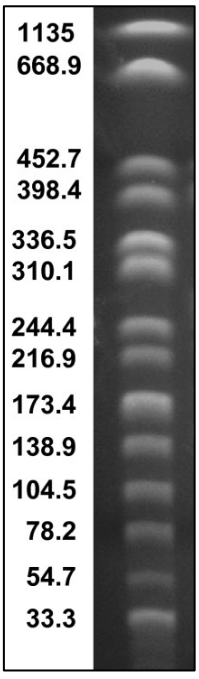
Product	Company
3 M NaCl, pH 7.0	Sigma-Aldrich, Steinheim, D
0.3 M sodium citrate	Sigma-Aldrich, Steinheim, D

1X Tris borate EDTA (TBE) buffer

Product	Company
90 mM Tris-borate, pH 8.0	Sigma-Aldrich, Steinheim, D
2 mM EDTA, pH 8.0	Sigma-Aldrich, Steinheim, D

1X Tris EDTA (TE) buffer

Product	Company
10 mM Tris-HCl, pH 8.0	Sigma-Aldrich, Steinheim, D
1 mM EDTA, pH 8.0	Sigma-Aldrich, Steinheim, D



Salmonella enterica serovar Braenderup PFGE size marker

The universal *Salmonella* PFGE size standard for all *Salmonella* strains, was *Xba*I-digested serovar Braenderup DNA (Fig. 3). All PFGE reactions were performed under the standardised electrophoresis conditions (see 2.2.9) specific for *Salmonella*.

Figure 3: PFGE *S. enterica* serovar Braenderup reference standard (H9812) used for *Salmonella* strains. Approx. kilobases band sizes, used for normalisation, are indicated (copied from Hunter et *al.* (112)).

2.1.6 Reaction kits

BioPrime Plus Array CGH Genomic Labelling System (Invitrogen)

Kit components	**Composition**
1.5 mL amber reaction tubes	
10X nucleotide mix with 5-aminohexylacrylamido-dCTP	10 mM Tris-HCl and 1 mM EDTA, pH 8.0
Binding buffer B2	12.5 mM $MgCl_2$, pH 7.5, 40% (v/v) 2-Propanol
DNA purification spin columns with collection tubes	2 ml tubes
Elution buffer	10 mM Tris-HCl, pH 8.5
Panomer 9 solution	5'-end labelled with AlexaFluor555 or AlexaFluor647[a]
Reaction buffer 2.5X	125 mM Tris-HCl, 12.5 mM $MgCl_2$, pH 7.5
Stop buffer	0.5 M EDTA, pH 8.0
Washing buffer W2	80% (v/v) Ethanol[a]

[a] proprietary composition

DNeasy Blood and Tissue Kit (Qiagen)

Kit components	**Composition**
Buffer AL	50% (v/v) Ethanol[a]
Buffer ATL	pH 8.3[a]
Buffer AW1	75% (v/v) Ethanol[a]
Buffer AW2	80% (v/v) Ethanol[a]
Buffer AE	10 mM Tris/HCl, 0.5 mM EDTA, pH 9.0
Proteinase K	600 mAU/mL

[a] proprietary composition

2.1.7 Primers and Oligonucleotides

Microarray oligonucleotide probes were synthesized at 40 nmol scale with a C6-aminolink modification (Metabion AG, Munich, Germany), the oligonucleotides were desalted and monitored by MALDI-TOF mass spectrometry resulting in close to 100% modified oligonucleotides and a fraction of 34-58% full length oligonucleotides (Appendix I, Table A2). A 100 pmol/µL stock solution has been prepared.

Other oligonucleotides for PCR have been purchased as Custom Oligonucleotides, SePop desalting purified (Eurogentec, Deutschland GmbH, Köln, D) (Appendix I, Table A1).

2.1.8 Software

- Array Designer ver. 4.1 (Premier Biosoft, Palo Alto, CA, USA)
- BioNumerics ver. 5.1 (Applied Maths, Sint-Martens-Latem, Belgium)
- CorelDraw ver. 13 (Corel Corporation, Ottawa, ON, Canada)
- GenPix Pro ver. 6.1 (Molecular Devices, Sunnyvale, CA, USA)
- Microsoft Office Edition 2003 (Microsoft, Redmond, WA, USA)
- Microsoft Windows NT 4.1 & Windows XP SP3 (Microsoft, Redmond, WA, USA)
- QArray Mini Print program (Genetix, New Milton, UK)
- Reference Manager ver. 11 (Thomson Reuters, Carlsbad, CA, USA)
- SPSS ver. 12 (SPSS Inc., Chicago, IL, USA)

2.2 Methods

2.2.1 DNA Microarray production

2.2.1.1 Oligonucleotide design

The DNA microarray oligonucleotide probes were designed using the program Array Designer (version 4.1, Premier Biosoft, Palo Alto, CA). Relevant open reading frame sequences were selected from Genbank 166.0 (http://www.ncbi.nlm.nih.gov/Genbank/), imported in Array Designer and a cross homology analysis against the genome sequence of strain *S. enterica* subsp. *enterica* serovar Typhimurium strain LT2 (Accession no. NC_003197) was performed. Based on the avoidance of cross homologies, 57-60mer oligonucleotides were designed using the recommended default options for 60mer oligonucleotides with 73°C ± 5°C melting temperature, max. 6.0 [-kcal/mol] ΔG hairpin, max. 8.0 [-kcal/mol] ΔG self dimer and a maximal length of runs/repeats at 5 nucleotides. For five probes a shorter oligonucleotide length was selected because default options were not successful in finding probes, all other settings were not changed. The probes were assigned to seven different marker groups depending on their functionality of the corresponding gene sequence (number of probes): pathogenicity (83), resistance (49 probes), serotyping (33), fimbrial (21), DNA-mobility (57), metabolism (21), and prophages (13). Detailed information for each probe can be found in Table A2 (Appendix I).

In addition, three 60mer oligonucleotides derived from the *Arabidopsis thaliana* genes; RCA (M86720), RCP1 (NM_12175) and PRKASE (X58149) were designed used as negative control probes on the microarray.

2.2.1.2 Preparation of the source plate

The source plate should be prepared in a room free of *Salmonella* DNA. Pipette tips with filters were exclusively used. Cross-contamination of the samples was avoided carefully. During pipetting the reagents especially the enzymes were kept on ice. In order to avoid fluids on the reaction tube wall the tubes were spinned shortly in a micro-centrifuge before use. A 6-mL aliquot print buffer was diluted with 2.4 mL double distilled water. The 384-well microarray plate was filled with 25.2 µL per well using a multi-channel pipette. A 10.8-µL 100 mM oligonucleotide probe aliquot was added each using a multi-channel pipette. For using multi-channel pipettes, oligonucleotide probes were ordered in 96-deep well microtiter plates. A 9-µL aliquot of double distilled water in those wells which does not contain oligonucleotide probes.

2.2.1.3 Print process

The C6-aminolink oligonucleotides were printed on CodeLink activated slides (GE Healthcare, Munich, Germany) in a concentration of 30 µmol using a QArray Mini Arrayer (Fig. 4). As printing buffer 100 mM sodium phosphate, pH 8.5, was used. Per slide two array fields were printed (see Fig. 5). Each array consisted of two subarrays representing a duplicated set of probes. One subarray contained eight blocks (six columns and eight rows). The last row of each block contained probes representing positive controls (targeting the *ttrC* gene), negative controls (targeting three different *Arabidopsis thaliana* genes) and spotting buffer. The diameter of all spots was approx. 130 µm. The post-microarray blocking procedure was performed according to the manual instructions provided with the CodeLink activated slides. DNA microarrays were stored in a desiccator at room temperature and used within three months.

Figure 4: Qarray Genetix mini printer and humidity control station (Friedmann-Marohn).

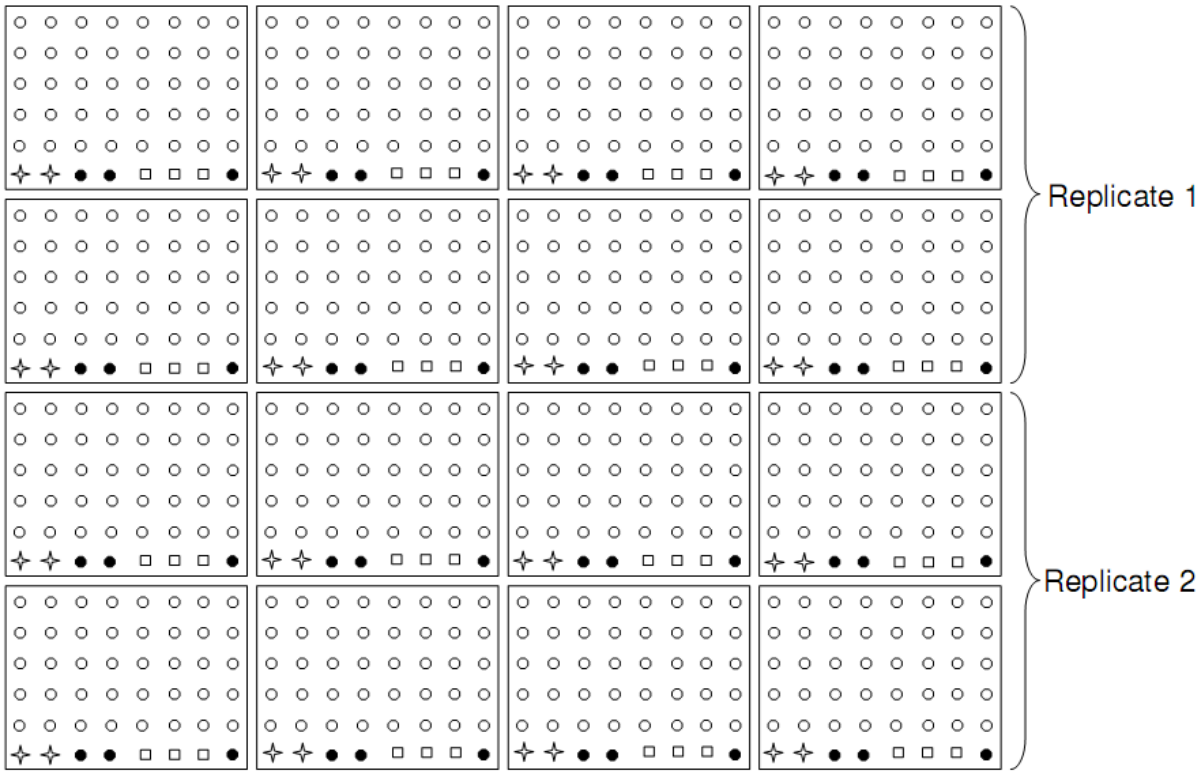


Figure 5: *Salmonella* DNA microarray schematic over view. The array consists of 16 blocks. Each block harbours a control row to the very bottom with two positive controls each (stars), three print buffer contamination controls (black circles) and three negative controls (boxes). The white circles in the upper five rows contain the oligonucleotide probes. First two rows of boxes represent one replicate of the probe set followed by a second replicate set; each probe is printed twice.

The slides were placed onto the slide holder. The activated surface must be placed on top. The print chamber and slide surfaces were kept free from dust. The humidity was kept between 25% and 50%. Eight 75 µm tungsten aQu split-pins (K2801; Genetix; Hampshire, UK) were loaded in the print head and the head was adjusted for 0.5 ± 0.2 mm inking depth. The source inking order was set by rows.

The printing program was adjusted as follows:

1. For slide design 8-pins/7-fields order and arraying by fields was selected. The field layout used fields no. 1, 2, 5 and 6. One field was divided in 8 blocks (8 columns, 6 rows) representing a full set of probes (*see* Fig. 5). All fields had the same probe assignment. Consequently, one slide contained two arrays; fields 1 and 2 formed the first array, fields 5 and 6 the second array.
2. The pattern dimension was set to 160 µm estimated spot size, row count 6, column count 8, row pitch 750 µm column pitch 500 µm.
3. The number of blots required before printing on the sample slides was set to 5 and the blot pitch was set to 650 µm.

4. The washing program between oligonucleotide inking was set to 3,000 ms washing using distilled water and 500 ms waiting. This washing step was repeated six times. The final step was 3,000 ms washing, 35,000 ms drying with compressed air and 5,000 ms waiting. The compressed air was totally clean, propellants and oil aerosols were avoided. Propellants influence the surface tension which influences the spot size and would have generated extremely large spots.
5. The slides were printed using 24 stamps per inking. The stamp time was set to 10 ms and inking time to 2 s.
6. After printing, the slides were placed immediately in a sealed chamber (e.g. desiccator with slide rack) containing a saturated sodium chloride solution at the bottom. The desiccator was closed, filled with pure oxygen and sealed with parafilm. The slides were incubated in this chamber overnight at room temperature.

2.2.1.4 Post-coupling processing

Residual reactive groups on the printed slides were blocked using 400 mL preheated blocking solution at 50°C for 30 minutes (min) with gentle shaking in a sealed chamber. The blocking solution was prepared without adding ethanolamine, adjusted to pH 9. Ethanolamine was added directly before use. The slides were dipped in distilled water once and afterwards the tray carrying six slides was loaded in a second incubation chamber filled with 400 mL of preheated post-print washing solution. The slides were incubated for 30 min at 50°C with gentle shaking. The slides were dipped in distilled water once and the tray was loaded in a third incubation chamber containing preheated distilled water. The slides were incubated at 50°C for 30 min with gentle shaking. For drying four slides were placed in a slide holder tube and centrifuged upright for 3 min at 5,000 x *g* (4,000 rpm). Until use the slides were stored at room temperature protected from light and humidity.

2.2.2 Salmonella *DNA purification*

For the purification of *Salmonella* DNA the DNeasy Blood and Tissue Kit (Qiagen) was used. A 1.6-mL aliquot of a *Salmonella* culture incubated for 16-18 h at 37°C in Luria-Bertani liquid medium was transferred into a sterile 2.0 mL reaction tube. The tube was centrifuged at 10,000 x *g* for 4 min. Pipette tips with filters were exclusively used. Cross-contamination of the samples was carefully avoided. During pipetting the reagents especially the enzymes were kept on ice. In order to avoid fluids on the reaction tube wall the tubes were spinned shortly in a micro-centrifuge before use. The supernatant was carefully discarded and the pellet

completely in 180 µL ATL buffer resuspended by vortexing. 25 µL Proteinase K was added and briefly mixed by vortexing. The suspension was incubated at 56°C for 3 hours using a thermo mixer at 750 rpm. After the incubation the tube was centrifuged upright for 10 s. The tube was cooled down at room temperature to approximately 40°C and 5 µL RNAse A (100 mg/mL) was added, briefly mixed by vortexing and incubated for 5 minutes at room temperature. The suspension was mixed by vortexing for 15 s and 210 µL AL buffer was added and mixed thoroughly by vortexing. 210 µL ethanol *p.a.* (96-100%) was added and immediately mixed by vortexing to yield a homogenous solution. The mixture was transferred into the DNeasy Mini spin column placed in a 2 mL collection tube. It was centrifuged at 10,000 x *g* (8,000 rpm) for 1 min. The collection tube was discarded and the column placed into a new collection tube. 500 µL AW1 buffer was added and the column was centrifuged at 10,000 x *g* (8,000 rpm) for 1 min. The flow-through was discarded the column was placed in a new collection tube. 500 µL AW2 buffer was added and the column was centrifuged at 10,000 x *g* (8,000 rpm) for 1 min. To dry the DNeasy membrane the column was centrifuged for 3 min at 17,500 x *g* (14,000 rpm). The flow-through was discarded and the DNeasy Mini spin column placed in a 1.5 mL micro-centrifuge tube. 50 µL AE buffer was added directly onto the DNeasy membrane. The column was incubated at room temperature for five minutes and centrifuged at 10,000 x *g* (8,000 rpm) for 1 min to elute DNA. The elution step was repeated and the column was again centrifuged at 17,500 x *g* (14,000 rpm) for 2 min. The eluted DNA was stored at 4°C until fluorescence labelling.

2.2.3 Fluorescence-labelling of genomic DNA

For the labelling of the genomic *Salmonella* DNA the BioPrime Plus Array CGH Genomic Labelling System was used.

General requirements for the labelling according to standard laboratory praxis have to be considered. Solely pipette filter tips were used. Cross-contamination of the samples was carefully avoided. All reagents, especially the enzymes, were kept on ice at all times. In order to avoid fluids on the reaction tube wall the tubes were spinned briefly in a micro-centrifuge before use. After adding fluorophores, the solutions were carefully protected from light for the whole process; especially from UV light wich would have bleached and lowered the signal intensity. Amber reaction tubes were used.

2.3.3.1 DNA labelling

Approximately 4 µg *Salmonella* genomic DNA in a maximum volume of 24 µL was filled in an amber 1.5 mL micro-centrifuge tube and the volume was adjusted to 24 µL with sterile water. Amounts of at least 4 to 10 µg genomic DNA were used but not more than 10 µg. A 20-µL aliquot of fluorophore-random oligonucleotide mix (Panomer 9 resuspended in Reaction Buffer) was added. The suspension was mixed by gentle vortexing and briefly centrifuged to collect the content. The mixture was incubated for 10 min at 95°C, immediately cooled on ice and protected from light. On ice, a 5-µL aliquot of 10X fluorophore nucleotide mix with AlexaFluor555-aha-dCTP or AlexaFluor647-aha-dCTP and 1.5 µL exo-Klenow fragment was added. Generally, AlexaFluor555-aha-dCTP was used for labelling. It was observed that this fluorophore labels DNA more efficiently resulting in stronger signal intensities than using AlexaFluor647-aha-dCTP. Differences in the specificity of both fluorophores were not observed. Again, the suspension was mixed gently but briefly by vortexing and then centrifuged to collect the contents. For the labelling reaction the tube was incubated at 37°C for 3.5 h in a water bath protected from light. To stop the labelling a 5-µL aliquot of stop buffer was added to the tube. The tube was place on ice.

2.2.3.2 Purification of labelled DNA

For purification, 200 µL binding buffer B2 was added to the labelled DNA and briefly mixed by vortexing. The sample was loaded onto the PureLink Spin Column placed in a 2 mL collection tube. The column was centrifuged at 10,000 x *g* (8,000 rpm) for 1 min. The flow-through was discarded and the column placed in a new collection tube. A 650-µL aliquot of wash buffer W1 was loaded onto the column. The column was centrifuged at 10,000 x *g* (8,000 rpm) for 1 min. The flow-through was discarded and the column was placed in a new collection tube. To remove any residual wash buffer the column was centrifuged at 17,500 x *g* (14,000 rpm) for 3 min. The column was placed in a new sterile amber 1.5 mL collection tube. A 55-µL aliquot elution buffer E1 was loaded on the column membrane and incubated at room temperature for 5 min. To collect the labelled DNA the column was centrifuged at 17,500 x *g* (14,000 rpm) for 2 min. The flow-through contained the purified labelled DNA. The eluate was dried in a Vacuum Concentrator at 60°C for 25 min.

2.2.4 Construction of an internal hybridisation control

In order to identify the absence of individual probes on the array field potentially caused by print errors an internal hybridisation control (IHC) was constructed. The 281 probes were added in low concentrations to the hybridisation solution showing presence of probes on the array by binding their spotted self's. A 2-µL (100 µmol) aliquot of all 281 probes printed on the array was pooled. Of this mix a 24-µL aliquot was labelled with AlexaFluor647 according to the protocol described above. The dried eluate was resuspended in 300 µL hybridisation buffer and a 0.8-µL (4 pmol) aliquot was used for the hybridisation.

2.2.5 Microarray hybridisation of the labelled DNA

The labelled DNA was protected from light at all steps as efficiently as possible. Pipette tips with filters were exclusively used. Cross-contamination of the samples was carefully avoided. During pipetting enzymes and fluorophores were kept on ice. In order to avoid fluids on the reaction tube wall the tubes were spinned briefly in a micro-centrifuge before use.

2.2.5.1 Hybridisation

The slide containing the printed probes was placed into the hybridisation chamber. One lifter slip per array field was placed onto the slide. A 30-µL aliquot of sterile water was added into humidity wells. The closed chamber was preheated at 42°C for 10 min. A 30-µL of prewarmed hybridisation buffer was added to the labelled and dried DNA. The dried DNA was resuspended by careful up- and down pipetting while air bubbles were avoided. The suspension was incubated at 95°C for 2 min. The sample was briefly centrifuged to collect the content. The prepared hybridisation chamber was opened and the sample carefully loaded under the lifter slip while air bubbles were avoided. The hybridisation chamber was closed and incubated at 42°C for approximately 18 hours in a water bath.

2.2.5.2 Post hybridisation washing

After incubation, the hybridisation chamber was opened and the slide was removed from the chamber with tweezers. In order to remove the lifter slips the slides were immediately rinsed in 300 mL of wash solution I preheated to 34°C. The slides were placed in a slide holder and incubated at 34°C for 3 min in wash solution I with gentle shaking. The slides were dipped once with 34°C preheated wash solution II and washed in a second incubation chamber at 34°C for 3 min in wash solution II with gentle shaking. The slides were dipped

once with 34°C preheated wash solution III and washed in a third incubation chamber at 34°C for 3 min in wash solution III with gentle shaking. The slides were dipped in sterile water at room temperature, placed in a slide holder tube (upright placed) and centrifuged at 5,000 x *g* (4,000 rpm) for 3 min. The slides were stored until scanning and kept dry and protected from light at room temperature.

2.2.6 Data handling and DNA microarray analysis

2.2.6.1 DNA microarray slide scanning

For a prescan the slide was introduced into the scan tray of the microarray scanner according to the manufacturer instructions. The Cy3 green laser light channel was selected for DNA labelled with AlexaFluor555 and the Cy5 red laser light channel was selected for DNA labelled with AlexaFluor647. Usually, the Photomultiplier (PMT) gain of both channels was set to 600. Positive control spots (*ttrC* probe) were used for defining optimal signal intensity. Pixels with intensities out of the range were strongly avoided since it was not an accurate measurement of the pixel intensity.

The array field was defined and a full scan with high resolution (10-µm pixel resolution) was performed. The hybridised slides were scanned with a GenePix 4000B laser scanner (Axon, Foster City, CA) using a resolution of 10 µm. Fluorescent images were captured as multi-image-tagged file format and analysed with the GenePix Pro 6.1 Software (Molecular Devices, Sunnyvale, CA, USA). An automatically-align-signal for identifying and analysing individual features was operated using a GAL (GenePix Array List) file and the feature intensities were quantified using the GenePix Pro software. A GAL file defined an array of blocks to match the size and positioning of printed features and to apply substance names to the features. The file can be usually generated by the microarrayer software. The local background intensity was subtracted from each feature's intensity. The raw feature and background intensities were saved as text files and these files were imported for normalisation in a table calculation program (i.e. MS Excel).

2.2.6.2 Normalisation of signal intensities

The signal intensity average was calculated from the two positive control spots (*ttrC* probe) of each block. For normalisation of the AlexaFluor555 probe signals detectable in the Cy3 channel of the scanner a ratio has been calculated as follows. The local background subtracted median spot intensity of each probe was divided by the local background

subtracted median spot intensity of the positive control probe identifying the *ttrC* gene, which is present in all *Salmonella enterica* subspecies *enterica* strains (103). The probe control detectable in the Cy5 channel of the scanner was used to highlight absent (e.g. not printed) probes. Probes lacking an AlexaFluor647 fluorescence signal below 100 units were considered to be print errors and were excluded from the analysis. Based on the average of the spot intensities of all negative target probes for *S. enterica* serovar Typhimurium strain LT2 probe signals for which the ratio was equal to or greater than 0.25 were considered as positive. Ratio values between 0.25 and 0.15 were classified as "uncertain". In this case individual decisions had to be made. The signal intensity depended on several factors e.g. the DNA quality, the hybridisation reaction as well as the labelling reaction. Moreover, the cut off value may differ between different chips with different surfaces and fluorophores. A normalised ratio below 0.15 was considered as target sequence absent. For the *E. coli* reference strain EC227 an artificial value of 10,000 unit's AlexaFluor555 fluorescence raw intensity for the *ttrC* probe was applied for the normalisation because the *Salmonella* specific *ttrC* probe gave no signal.

2.2.6.3 Analysis of microarray results

Normalised presence/absence data of each strain were imported in BioNumerics (version 5.1; Applied Maths, Sint-Martens-Latem, Belgium) as character values. Certain target marker groups (e.g. pathogenicity) were selected and a maximum parsimony tree calculation was performed to visualize the differences in the gene set between the strains tested (*see* Fig. 11). The cluster calculation analysis was performed with the simple matching binary coefficient using the unweighted-pair group method with arithmetic averages (UPGMA dendrogram type). The maximum parsimony cluster analysis was performed with 1,000 bootstrap cycles and the exported rendered tree was performed with hidden branches and distance labels shorter or equal to 1 and rooted tree type.

2.2.7 Validation of the DNA microarray

The introduction of PCR in the mid 1980s by Mullis and Faloona (168) has revolutionised molecular diagnostics. Because of speed, accuracy and automation PCR was in the last two decades increasingly applied to identify and to characterise various genetic targets including genes that are e.g. associated with virulence, antimicrobial resistance, or metabolism. This gene-detection profiling generates valuable data which can be used for the differentiation between *Salmonella* isolates but also in combination with epidemiological data to estimate the

potential risk for animals and humans. Using PCR, it is possible, in contrast with restriction enzyme digestion-based methods such as PFGE, to obtain data on the gene repertoire and biological properties of a strain. PCR has been frequently used to screen a large number of virulence and antimicrobial resistance genes (88).

PCR is a three step cyclic in-vitro procedure based on the ability of DNA-polymerase to copy a strand of DNA. When two primers bind to complementary strands of target DNA, the sequence in between is amplified exponentially in presence of deoxynucleoside triphosphate (dNTP's) with each cycle, making the technique a very sensitive tool. The choice of the primer sequences determines the specificity, and consequently the success, of the PCR. A reaction requires on average 1.5 to 2 hours of thermal cycling followed by detection of the PCR products. The reaction, commonly carried out in a thermal cycler and a reaction volume of 10-200 µl, starts with a short, approx. 5 minutes long denaturation step at 95°C separating the double-stranded DNA in single-stranded DNA. Afterwards the primers anneal at lower temperatures, usually at approximately between 50 and 60°C. The strand elongation takes place at optimal DNA-polymerase temperatures at 72°C. After 30 to 40 repeated cycles, the PCR product is electrophoretically separated in an agarose gel and the fragments visualized by staining the gel with ethidium bromide.

2.2.7.1 Validation of the microarray signals by PCR

PCR's were performed for the target genes indicated in supplementary material (Appendix I, Table A1). PCR primers were designed by the Array Designer 4.1 (Premier Biosoft, Palo Alto, CA) and resulted in 400-500 bp amplification products. For the detection of the most antibiotic resistance genes, published primer sequences from various sources were used. A complete list of PCR primers, PCR product sizes, references and their characteristics is available in supplementary material (Appendix I, Table A1). A typical 25-µL PC-reaction is shown in table 7. The identical DNA preparation used for the AlexaFluor555-labeling of each strain was used for the PCR reaction. The incubation conditions were 95°C for 1 min, followed by 33 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s. A 10-µL aliquot of a PCR product was loaded on a 1.5% agarose gel and an electrophoresis at 6 V cm^{-1} for 90 min was performed. After electrophoresis the gel was stained in ethidium bromide solution for 10 min and photographed under UV-light using the EagleEye-II gel documentation system (Stratagene, USA). The presence of a clear fragment with the correct amplification size has been assessed as a positive signal (presence of the gene).

Table 7: Exemplary PCR reaction

µL/PCR	Component and concentration
1	Primer 1 (10 pmol/µL)
1	Primer 2 (10 pmol/µL)
2.5	d'NTP Mix (200 µM final conc. per nuc.)
2.5	10X PCR buffer
0.75	50 mM $MgCl_2$ (final conc. 1.5 mM)
0.2	*Taq* Polymerase (5U/µL)
12.05	double-distilled water
5	Template-DNA
25	

2.2.7.2 Evaluation of probe specificity

For the assessment of the probe specificity, nucleotides within the positive control oligonucleotide probe sequence detecting the *ttrC* gene in *Salmonella* species were exchanged at various positions and with different similarities (Table 8). Nine different polymorphic patterns were designed, including continuing 16-mer nucleotide exchanges at different positions of the sequence and single nucleotide exchanges distributed over the complete 60-mer sequence with homologies of 70%, 80%, and 90%. Nucleotide changes did not modify the melting temperature of the original probe sequence. The set of polymorphic oligonucleotides was printed on CodeLink-activated slides. AlexaFluor647-labeled serovar Typhimurium DNA (strain 51K61) has been hybridised according to the protocol described above, and a mean ratio value of the spot intensities for each polymorphic probe sequence compared to the 100% complementary *ttrC* probe sequence based on eight independent experiments has been calculated as described above.

Table 8: Oligonucleotide probe variations of *ttrC* gene sequence in *S. enterica* serovar Typhimurium

Name	Sequence[a]	% Similarity
ttrC	ATGACGCATTCACTCATCATTGAAGAAGTGCTGGCTCACCCGCAGGACATTAGCTGG	100
ttrC2	**TACCTTGGAAGGTAGT**TCATTGAAGAAGTGCTGGCTCACCCGCAGGACATTAGCTGG	72
ttrC3	ATGACGCATTCACTCATCATTGAAGAAGTGCTGGCTCACCC**AGGCTTGCAAGCTACC**	72

ttrC4	ATGACGCATTCACTCATCATT**CTTCTTCACGACCGAG**ACCCGCAGGACATTAGCTGG	72
ttrC5	AT**CAG**GCAA**T**G**A**C**A**C**T**TCATA**G**T**A**GA**T**GTG**GT**C**G**C**A**GAC**G**CGCA**C**GA**G**ATTA**C**CTC**G**[b]	70
ttrC6	AT**C**ACG**G**ATTC**T**CTCAT**G**ATTGT**A**GAAGA**G**CTCGC**T**CAC**G**CGCAC**G**ACATTAC**C**TGC[b]	81
ttrC7	ATGACGCAATCACTCAT**G**ATTGAAGAA**C**TGCTGGCTC**T**CCCGCAGGA**G**ATTAGCTG**C**[b]	89
ttrC8	ATAACTCA**G**TC**G**CTAATTATCGAC**G**ACGTA**C**TGGC**G**CACACGTAG**T**AC**G**TTC**G**CC**G**G[c]	70
ttrC9	ATGA**T**GCAT**G**CACTAATCA**G**TGAAT**A**AGTA**C**TGGATCAC**T**CGC**C**GGAC**G**TTAGC**C**GG[c]	81
ttrC10	**C**TGACGCA**G**TCACTCATAATTGAAGAA**T**TGCTGGC**T**CGCCCGCAG**G**ATATTAGCTGG[c]	89

[a] Nucleotide changes to the original ttrC probe sequence are indicated in bold
[b] Purine (A, G) bases in nucleotides were exchanged by pyrimidine (T, C) bases
[c] Pyrimidine bases in nucleotides were exchanged by purine bases

2.2.8 Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) is currently the method of choice for molecular sub-typing *Salmonella* serovars. Since its initial description in 1983 (215) it has been proven to be a useful discriminatory method and was standardised by the PULSE-NET Consortium (1,178,184). To perform PFGE, approx. 10^8 cells are embedded in an agarose matrix. The agarose plugs are treated with detergents and enzymes to lyse the embedded cells and to release the DNA. The DNA is treated with a rare cutting restriction enzyme, such as *Xba*I or *Bln*I. The plug containing the restricted DNA is inserted into the well of an agarose gel and the DNA fragments are separated under electrophoretic conditions of alternating polarity. This pulsed field enables the separation of very large DNA fragments (10 to 800 kb) (214). Following electrophoresis, the pattern of DNA fragments is visualized by staining the gel with ds-DNA intercalating dye, e.g. ethidium bromide. Resulting fragment patterns can be compared between different isolates by commercial software e.g. BioNumerics (Applied Maths). With the use of analysis software it is possible to create a PFGE pattern databank necessary to analyse strains according their relatedness. A classification scheme of genetic relatedness based on PFGE patterns had been proposed by Tenover *et al.* (229). Four categories, namely: indistinguishable, closely related, possibly related and different from the outbreak pattern have been identified (229). Closely related PFGE patterns differ by a single genetic event resulting in e.g. a shifted fragment or two smaller fragments instead of one larger fragment. These events may be caused by a point mutation, an insertion or deletion leading to a fragment number between one and three. Often, one additional fragment with a

size between 20 and 50 kb indicates the presence of a plasmid in the strain. Recommendations for the interpretation of PFGE patterns including *Salmonella* have been updated by Centres for Disease Control and Prevention (CDC) because the Tenover *et al.* criteria did not adequately account for differences associated with genetic events, such as horizontal gene transfer (200). The interpretation is more dependent on the natural history of the species being typed rather than using just the number of differences in fragments and their locations. Temporal and geographical information should be included in the interpretation. A similar pattern detected in a wide space of time and/or in diverse geographical areas may not be the same strain as an isolate that is associated with an ongoing outbreak (200).

Owing to the standardisation efforts by PULSE-NET an advantage of PFGE is that it is highly reproducible allowing for data sharing among multiple laboratories. The genetic profile is based on the entire genome. Most investigators have good success in molecular typing of isolates belonging to one serovar (98,143).

Pulsed-field gel electrophoresis (PFGE) has become the "gold standard" among other molecular high precision typing methods (1,178) representing the method of choice for genetic relatedness studies among *Salmonella* strains. Bacterial DNA is cleaved by rare-cutting restriction enzymes e.g. *Xba*I (Roche Diagnostics, Mannheim, Germany). The large, up to 2000 kb, fragments (214), obtained from the restriction are separated by a special electrophoresis technique performed according to the standardised PulseNet *Salmonella* protocol (248). The *S. enterica* serovar Braenderup reference standard H9812 restricted with *Xba*I was used size marker (see 2.1.5).

2.2.8.1 Preparation of DNA-agarose blocks

Until the preparation of DNA-agarose blocks all solutions were kept on ice. *Salmonella* strains were cultured for 20-24 h on LB-agar plates at 37°C. Three to four colonies were transferred to 4 mL cell suspension buffer by a sterile plastic loop. The optical density of this suspension was adjusted to 1.3-1.4 absorbance (610 nm). A 300-µL aliquot of the cell-suspension was mixed with 15 µL Proteinase K (final concentration 0.5 µg/µL), 1% (w/v) SDS and 300 µL 50°C heated agarose in 50°C pre-warmed 1.5 mL reaction tube. Directly after mixing the suspension was transferred into PFGE-reusable plug moulds while carefully avoiding bubbles. For solidification the moulds were kept for 30 min at 4°C.

2.2.8.2 Cell-lyses in DNA-agarose blocks

After solidification the DNA-agarose blocks were carefully removed from their plug moulds by a sterile applicator and transferred into 5 mL cell-lyses buffer in 15 mL FALCON tubes. Lysis was performed at 54°C for 2 h in a water bath by gentle shaking (approximately 200 rpm).

2.2.8.3 Washing of DNA-agarose blocks

The blocks were washed twice for 15 min at 50°C in a water bath by gentle shaking (approx. 200 rpm) using 15 mL pre-warmed (50°C) sterile, double-distilled water. Another washing step using 15 mL pre-warmed (50°C) TE buffer was performed four times. After washing the DNA-agarose blocks were cut into (approx.) 2.5 mm slices using a sterile scalpel and stored at 4°C in TE buffer in 15 mL FALCON tubes.

2.2.8.4 Digestion with Xba*I*

One slice was introduced in 120 µL of 1X SureCut H-buffer (Roche) and incubated for 15 min. The buffer was replaced by 100 µL new *Xba*I 1X SureCut H-buffer containing 0.25 U/µL restriction enzyme *Xba*I. The restriction was performed for 4 h at 37°C in a water bath. For stopping the restriction the solution was discarded and a 500-µL aliquot 0.5X TBE buffer was added.

2.2.8.5 Preparation of the PFGE gel

One slice each was placed on the comb teeth of a horizontally positioned PFGE comb using a sterile applicator. After 5 min incubation time for drying, the comb with the placed slices was introduced within a gel mould try. The preheated (50°C) agarose was introduced in the mould carefully avoiding the replacement of the slices. For solidification the gel was kept for 30 min at room temperature.

2.2.8.6 Performance of electrophoresis and staining

The PFGE chamber (CHEF-DR III, Variable Angle System, BioRad) was filled with 2.5 litres of 0.5X TBE running buffer containing 100 µM thiourea for prevention of DNA degradation. After filling the chamber the running buffer was cooled to 14°C (duration approximately 30 min). The comb was replaced and electrophoresis was performed for 20 h with 6V/cm (200 V), 2-64 seconds intervals of the current at 120° angle. After the

electrophoresis the gel was stained for 20 min in ethidium bromide solution (0.5 µg/mL) and destained for 20 min in sterile, double-distilled water. The gel was documented under UV-light by EagleEye-II gel documentation system (Stratagene, USA).

2.2.8.7 Cluster analysis

Obtained PFGE fragment profiles were analysed by BioNumerics 5.1 (Applied-Maths, Saint-Martens-Latem, Belgium). The comparison was performed by band matching using Dice coefficient with a 1.5% band tolerance and UPGMA dendrogram type. Strains were assigned to the same PFGE profile type when they showed visually the same fragment sizes between the marker fragment sizes of 1135 kb and 33 kb.

2.2.9 Antimicrobial susceptibility testing by minimal inhibitory concentration (MIC)

Susceptibility to 17 antimicrobials and antimicrobial mixtures were assessed by determining the minimum inhibitory concentration using the CLSI broth micro dilution method (131). An overnight culture grown at 37°C on Müller-Hinton (MH)-agar plate was taken by a sterile plastic loop and resuspended in 5 mL autoclaved saline solution (0.85% NaCl). The opacity was measured to achieve 0.5 McFarland in the Sensititer system (Autoinoculator INO2, Trek Diagnostic systems). A 30-µL aliquot was transferred into 11 mL MH II-broth (Beckton-Dickinson) and briefly mixed. A 50-µL aliquot was applied in each well of a microtiter plate (NLMV1A, Trek Diagnostic Systems) providing lyophilized antimicrobials (see below) in several concentrations in its wells. After filling the bacterial suspension into the wells of the microtiter plate, the plate was covered by an adhesive film and incubated at 37°C for 18 h. The growth of bacteria in the wells was read and analysed by a semi-automatic sensitouch system (Trek Diagnostic Systems). The MIC value was determined to the lowest antimicrobial concentration growing of cells was detectable. The obtained concentrations (in µg/mL) were compared with breakpoints assessed by the Clinical and Laboratory Standard Institute (CLSI), the European Committee of Antimicrobial Susceptibility testing (Eucast, www.eucast.org), the Antibiotic Resistance in bacteria of animal origin (9) and the Danish Integrated Antimicrobial resistance Monitoring and Research Programme (DANMAP 2001, http://www.danmap.org/) and concluded in susceptible (S), intermediate (I), and resistant (R). Antibiotics tested and susceptibility breakpoints (in brackets) were: AMC, amoxicillin/clavulanic acid (≤ 16); AMP, ampicillin (≤ 4); CHL, chloramphenicol (≤ 16); COL, colistin (≤ 2); CIP, ciprofloxacin (≤ 0.125), FLO, florfenicol (≤ 16); GEN, gentamicin (≤ 2), KAN, kanamycin (≤ 32); NEO, neomycin (≤ 8); NAL, nalidixic

acid (≤ 16); SPE, spectinomycin (≤ 64); STR, streptomycin (≤ 32); SMX, sulfamethoxazol (≤ 256); SXT, trimethoprim/sulfamethoxazol (≤ 2/ ≤ 38); TET, tetracycline (≤ 8); TMP, trimethoprim (≤ 2); XNL, ceftiofur (≤ 4).

3. Results

3.1 *Salmonella* DNA microarray development

The development of the DNA microarray for the characterisation and typing of *Salmonella enterica* subsp. *enterica* isolates included the design of the target specific probes and their validation by target specific PCR reactions. Additionally, the optimization of the production process including printing, optimization of the DNA isolation, optimisation of the array hybridisation and scanning procedures was performed. Only final data signal intensities and adjustments are shown in this thesis.

3.1.1 Construction of microarray and controls

Two hundred and seventy-six 57-60mer and five 40-45mer target specific oligonucleotide probes were designed for the molecular characterisation and typing of *Salmonella* isolates. The oligonucleotide probes target specific DNA sequences of genes which are associated with (i) the pathogenicity of *Salmonella* such as type 1 or type 3 secretion systems, outer membrane proteins, secreted proteins, Vi antigen encoding genes or virulence markers located in prophages, (ii) with antibiotic resistance determinants, (iii) with fimbrial clusters, (iv) with prophages, (v) with phase-1 (H1) and phase-2 (H2) flagellar antigens, (vi) with lipopolysaccharide (O) antigens, (vii) with IS elements, (viii) with plasmid incompatibility groups, and (ix) with metabolism (Appendix I, Table A2). The size of probes (57-60mers) was selected because in expression studies it has been described that 60mer oligonucleotides reliably detect transcript ratios (110).

Probes were printed using 100 mM Na_3PO_4 print buffer and 75 µm aQu split pins (Genetix) on CodeLink (Amersham) activated glass slides. The microarray-slide harboured two detached array fields each consisting of 16 blocks composing of 6 rows by 8 columns and altogether 768 spots each, enabling two separate experiments per slide (Fig. 5). Several controls were included for quality and print control reasons. Two spots per block positive control *ttrC* probe were built-in for normalisation of the raw signal intensities of each block (see 2.3.3). Moreover, three negative controls, *Arabidopsis thaliana* genes, were integrated. Three contamination controls were included as well, detecting insufficient washing processes.

3.1.2 Validation of the DNA microarray

For validation of the DNA microarray, 23 *Salmonella* reference strains and one *E. coli* reference strain were selected (Table 7). These strains represented positive and negative controls for all oligonucleotide probes printed on the microarray. Each strain was tested for presence/absence of the 281 oligonucleotide probe targets by hybridisation of AlexaFluor555-labelled genomic reference strain DNA to the microarray. The result for each probe was compared with a target-corresponding specific PCR amplification. Altogether primers for 256 target genes were designed or selected from literature for PCR screening (Appendix I, Table A1). Results of probes belonging to the serotyping marker groups (targeting *fliC*, *fljB*, *rfb* specific sequences) were phenotypically confirmed by serotyping according to White-Kauffmann-Le Minor scheme (186). All negative controls derived from three *A. thaliana* genes and spotting buffer, showed ratios below 0.1 (data not shown) and were consistently classified negative. As shown in Table 9 the comparison between microarray and PCR results obtained from the 23 *Salmonella* reference strains gave an agreement of 94.94% (6227 data signals). The remaining part included 3.51% (226 data signals) gave inconsistent PCR and microarray results, additionally 1.55% (100 microarray data signals) were classified as uncertain (Table S1, supplementary material on CD). The disagreements occurred primarily by probes linked to mobile elements (88 data signals). Over 60% (139 data signals) of differences between microarray and PCR data occurred owing to wrong negative PCR results.

Table 9: Microarray validation results of the 23 *Salmonella* reference strains

Comparison microarray to PCR results	No. of signals	% Ratio
Agreement positive signals	2687	41.72
Agreement negative signals	3427	53.22
Total	6114	**94.94**
Disagreement (PCR neg. / microarray pos.)	139	2.16 (61.5)
Disagreement (PCR pos. / microarray neg.)	87	1.35 (38.5)
Total	226	**3.51**
Total uncertain microarray signals	100	**1.55**
Total	6440	**100**

3.1.3 Evaluation of the specificity of oligonucleotide probes

For the assessment of the probe specificity nucleotides within the positive control, oligonucleotide probe sequence detecting the *ttrC* gene in *Salmonella* were exchanged at various positions and different similarity (see Table 8). The nine different polymorphic patterns designed, including continuing 16mer nucleotide exchanges at the left, middle and right position of the sequence and exchanges distributed over the complete 60mer sequence with homologies of 70%, 80% and 90%, resulted in reduced intensity ratios as shown in Table 10. The highest influence could be detected within the scattered polymorphic probes with similarities of 70% (ttrC5 and ttrC8). These probes resulted in intensities recognised as absent. Additionally three probes, ttrC6, ttrC7 and ttrC9 showed intensities with cut-off ratio values of approximately 0.25.

Table 10: Ratios resulting from *ttrC* oligonucleotide probe mutations in serovar Typhimurium

Name	Sequence[a]	% Similarity	Ratio[d] (± SD[e])
ttrC	ATGACGCATTCACTCATCATTGAAGAAGTGCTGGCTCACCCGCAGGACATTAGCTGG	100	0.991 ± 0.07
ttrC2	**TACCTTGGAAGGTAGT**TCATTGAAGAAGTGCTGGCTCACCCGCAGGACATTAGCTGG	72	0.423 ± 0.06
ttrC3	ATGACGCATTCACTCATCATTGAAGAAGTGCTGGCTCACCC**AGGCTTGCAAGCTACC**	72	0.713 ± 0.04
ttrC4	ATGACGCATTCACTCATCATT**CTTCTTCACGACCGAG**ACCCGCAGGACATTAGCTGG	72	0.853 ± 0.19
ttrC5	AT**C**A**G**GCA**A**T**G**AC**A**C**T**TCAT**A**G**T**AGA**T**GTG**G**T**C**GC**A**GAC**G**CGCA**C**GA**G**ATTA**C**CT**C**G[b]	70	0.061 ± 0.02
ttrC6	AT**C**ACG**G**ATTC**T**CTCAT**G**ATTG**T**AGAAG**A**GCT**C**GCTCAC**G**CGCA**C**GACATTAC**C**TG**C**[b]	81	0.259 ± 0.11
ttrC7	ATGACGCA**A**TCACTCAT**G**ATTGAAGAA**C**TGCTGGCTC**T**CCCGCAGGA**G**ATTAGCTG**C**[b]	89	0.268 ± 0.04
ttrC8	AT**A**AC**T**CA**G**TC**G**CT**A**ATT**A**TC**C**GA**C**GA**C**GT**A**CTGGC**G**CAC**A**CG**T**AG**T**AC**G**TT**C**GC**C**GG[c]	70	0.066 ± 0.02
ttrC9	ATGA**T**GCAT**G**CACT**A**ATCA**G**TGAA**T**AAGT**A**CTGG**A**TCAC**T**CGC**C**GGAC**G**TTAGC**C**GG[c]	81	0.257 ± 0.13
ttrC10	**C**TGACGCA**G**TCACTCAT**A**ATTGAAGAA**T**TGCTGGC**T**CGCCCGCAGGA**T**ATTAGCTGG[c]	89	0.575 ± 0.04

[a] Nucleotide changes to the original *ttrC* probe sequence are indicated in bold
[b] Purine bases in nucleotides were exchanged by pyrimidine bases
[c] Pyrimidine bases in nucleotides were exchanged by purine bases
[d] Mean ratio value for each probe sequence based on four independent duplicates
[e] SD = Standard Deviation

3.1.4 Internal hybridisation control

An internal hybridisation control (IHC) was developed in order to recognise false negative results (Fig. 6). These might occur because of insufficient hybridisation, intensity gradients, bubbles under the cover slip, lint or spotting errors. For preparation, all probes which have been printed on the microarray were each pooled in a concentration of 4 pmolar. The mix was labelled with dye AlexaFluor647, and added to the AlexaFluor555-labelled genomic DNA in the hybridisation buffer. During hybridisation, AlexaFluor647-labelled probe sequences and AlexaFluor555-labelled genomic DNA competitively binds to the complementary probe coupled to the surface of the microarray. Bound AlexaFluor647-labelled probes have been reliably detected in the red (Cy5) channel with an intensity of 200-300 raw units while bound AlexaFluor555-labelled genomic DNA signals have been detected in the green (Cy3) channel. Owing to competitive hybridisation with the IHC AlexaFluor555-labelled genomic DNA was lowered by approximately 25% in comparison to hybridisation without IHC (data not shown).

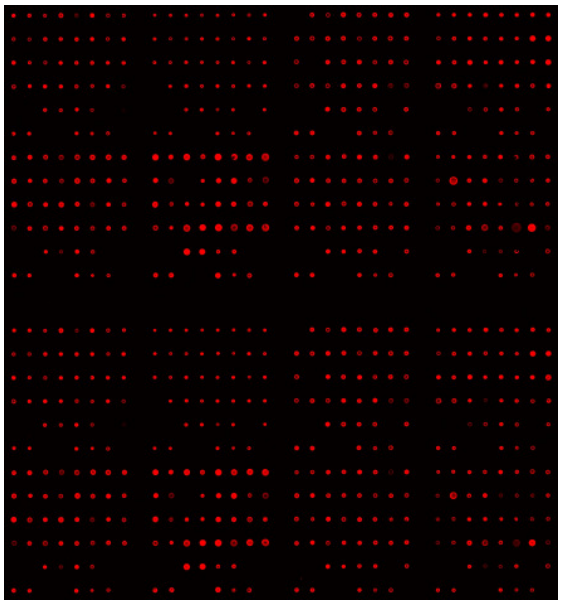


Figure 6: Internal hybridisation control. Microarray scan shows all 16 blocks in the Cy5 channel.

3.1.5 Experimental workflow

Figure 7 shows the developed DNA microarray experimental workflow. On the left side the DNA microarray production is shown. After probe design (Array Designer 4.1, Premier Biosoft) the external synthesised oligos (Metabion) were printed onto the coated slides. After blocking and washing steps the slides are prepared for hybridisation. *Salmonella* DNA was isolated from an overnight culture and labelled. After the 18 h hybridisation the slide was washed, scanned and raw data further analysed (Fig. 8).

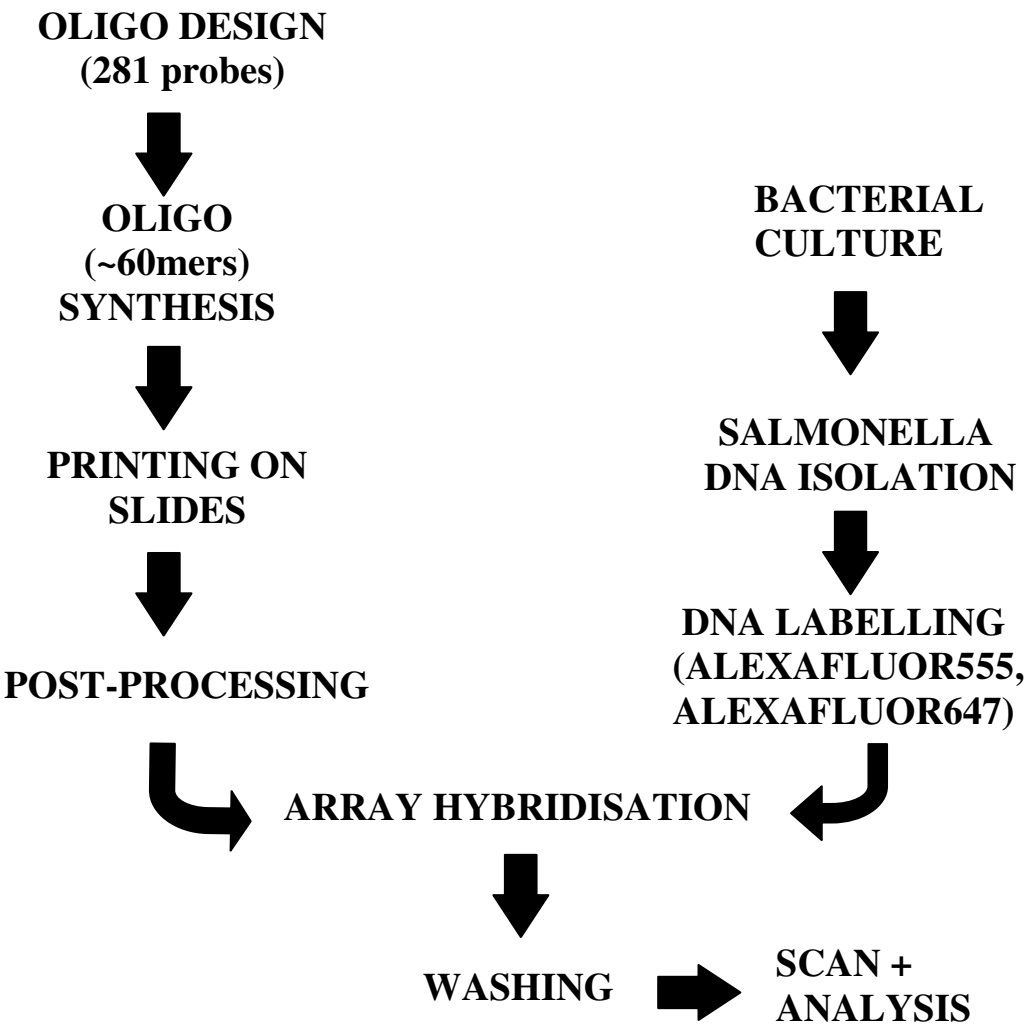


Figure 7: Experimental workflow of a DNA microarray experiment. Oligonucleotides are custom designed and synthesized; then printed on pre-coated slides and after post-processing labelled whole genomic bacterial DNA is hybridised, washed and afterwards scanned. The raw data further analysed.

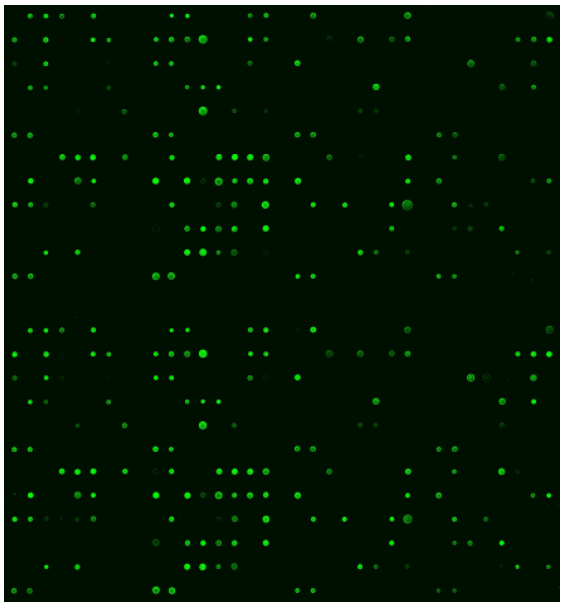


Figure 8: Example of a microarray analysis scan. All 16 blocks are shown in the Cy3 channel. Every green spot represents a present gene of the genome investigated.

3.2 Investigation of *S. enterica* subsp. *enterica* serovar 4,12:d:-

3.2.1 Analysis of serovar 4,12:d:- using PFGE.

Thirteen different *Xba*I PFGE profiles were recognised within 56 serovar 4,12:d:- strains analysed (Fig. 9). All profiles showed highly similar patterns. The most prevalent profile was PFGE profile 1 (19 strains) followed by PFGE profile 2 (9 strains) (Appendix I, Table A3). Compared to profile 1, the other profiles differed in one to five fragments. PFGE profile 1 was mainly found in broiler and feed isolates but also occurred in two of 17 isolates from humans investigated. PFGE profile 2 was found in one isolate from a broiler, in one from feed, in one from a pig, and in six from humans. More rarely, PFGE profile 3 was detected in isolates from broilers, turkeys, and humans. All serovar 4,12:d:- PFGE profiles showed low similarities to serovars Duisburg, Schwarzengrund, and Stanley, harbouring the same somatic (O) antigen and phase-1 flagellar (H1) antigens, but expressed in addition a phase-2 (H2) flagellar antigen, and serovar Derby expressed a variable H2 flagellar antigen (Fig. 9).

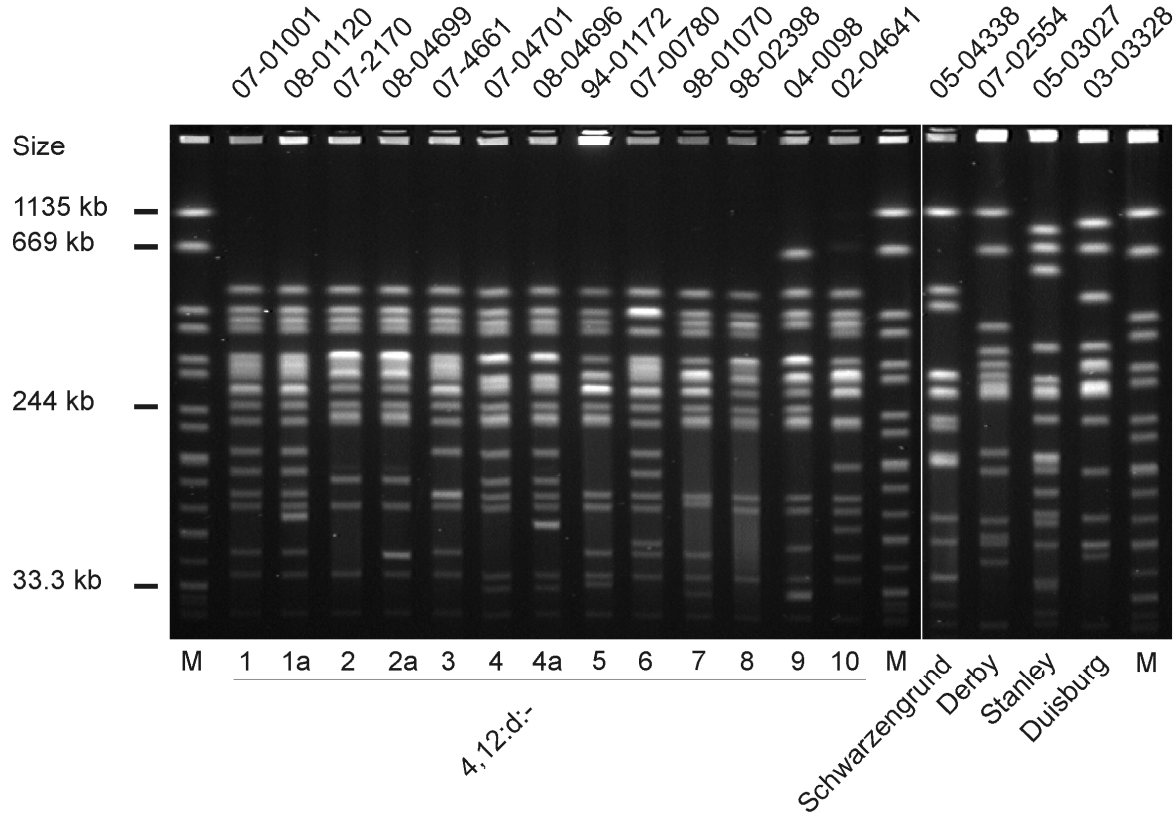


Figure 9: PFGE profiles of representative *S. enterica* subsp. *enterica* strains after digestion with *Xba*I. As molecular weight standard (Marker, M) *S. enterica* serovar Braenderup reference strain H9812 was used (Lanes 1, 15 and 20). Lanes 2 to 14 represent the 13 different PFGE types of serovar 4,12:d:-. Lanes 16 to 19 represent the PFGE patterns of selected strains and serovars possessing a related antigenic formula to serovar 4,12:d:- (serovar Schwarzengrund, serovar Derby, serovar Stanley, and serovar Duisburg). Above the gel strain numbers are indicated.

3.2.2 Virulence determinants characterisation of the serovar 4,12:d:-

Most *Salmonella* serovars can express two different antigenic flagella phases. The phase-2 flagellar locus is possibly used in particular environmental circumstances and host-defence mechanisms (160). Since the poultry associated serovar 4,12:d:- is monophasic it lacks this potential virulence determinant of *Salmonella* pathogenicity. The microarray results revealed, that all serovar 4,12:d:- isolates were monophasic because the phase-2 flagellar encoding region harbouring the regulatory genes *hin* and *fljA* as well as the *fljB* gene encoding the structural filament unit was consistently absent. In addition to investigating flagellar-related genes, the DNA microarray developed in this thesis was used to investigate 104 virulence determinants (83 pathogenicity and 21 fimbrial markers) of serovar 4,12:d:-. Among the 21 serovar 4,12:d:- strains, two virulence array types (VATs) were defined (Appendix I, Table A3). A new type has been assigned if the number of virulence determinants analysed by the microarray differed in more than one marker compared to the predominant virulence determinant pattern recognised in serovar 4,12:d:- strains. VAT 1 was with 90% (19 strains) the most prevalent type and found in all sources of isolates from feed, turkeys, chickens, pigs and humans (Fig. 10). VAT 2 was exclusively found in two feed isolates. VAT 2 differed to VAT 1 in two virulence determinants. In both VAT 2 strains, *trhH* encoding a pilus assembly protein and *srfJ* encoding a glucosyl ceramidase were present, whereas in the second VAT 2 strain *sopD2* was additionally absent.

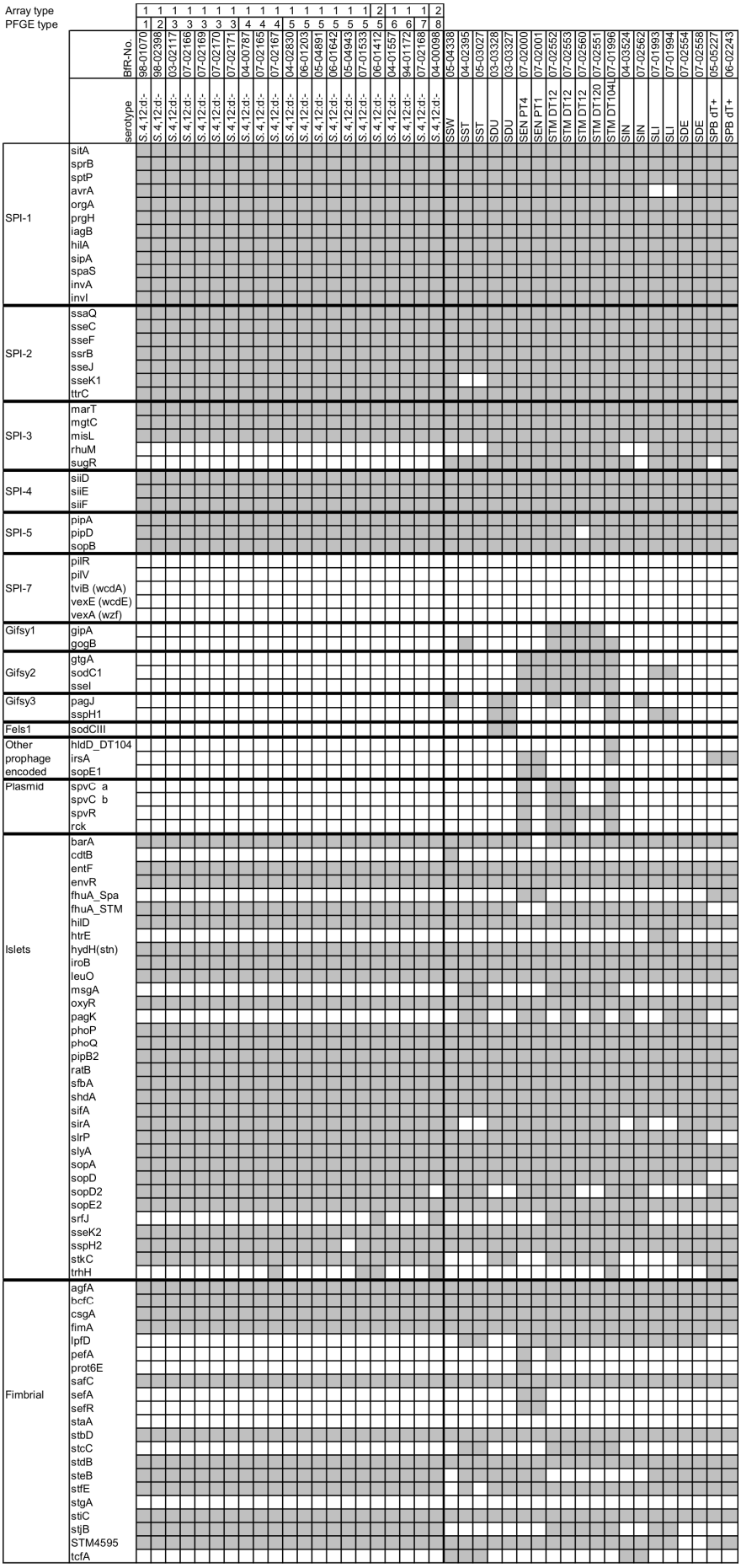


Figure 10: Virulence determinants microarray data of the 41 strains analysed. On top the strain numbers and serovars namely, SSW (serovar Schwarzengrund), SST (serovar Stanley), SDU (serovar Duisburg), SEN (serovar Enteritidis), STM (serovar Typhimurium), SIN (serovar Infantis) SLI (serovar Livingstone), SDE (serovar Derby) and SPB dT+ (serovar Paratyphi B dT+) are indicated. In addition, the PFGE type and the Virulence-Array type (VAT) of the serovar 4,12:d:- strains are shown. On the left side, the analysed genes are grouped according to their particular genomic location (*Salmonella* Pathogenicity Islands SPI-1 to SPI-7, Prophages Gifsy-1, Gifsy-2, Gifsy-3, and Fels-1; plasmids; islets) or function (fimbrial). The hybridisation result of a particular strain is shown by one column within the figure. A white box indicates the absence and a grey box the presence of the target sequence in the strain. BfR, Bundesinstitut für Risikobewertung

The following observations are remarkable in regard to the presence/absence of the virulence determinants: Virulence genes previously described to be located within prophages including Gifsy-1, Gifsy-2, Gifsy-3 and Fels-1, were absent in all isolates. Within SPI-3 the genes *rhuM* and *sugR* (3'-region of SPI-3) were absent in all serovar 4,12:d:- strains. This truncation has been formerly described for other serovars (27). The presence of a virulence plasmid as described for several serovars (45) that could not be recognised was confirmed by the absence of the genes *spvC*, *spvR*, *rck*, *pefA*, and *traT* and the incompatibility marker FIIs. Twelve out of 21 detectable fimbrial markers could be found in serovar 4,12:d:-. The long polar fimbriae (*lpfD*) usually found in serovar Typhimurium and serovar Enteritidis was absent.

3.2.3 Comparison of serovar 4,12:d:- virulence determinants to other serovars

The pathogenicity markers of serovar 4,12:d:**-** were compared to those of various other serovars expressing the same O-antigens or commonly associated with poultry. Three of the serovars, Duisburg (4,12:d:e,n,z_{15}), Stanley (4,12:d:1,2) and Schwarzengrund (4,12:d:1,7), share the same O- and H1-antigen with serovar 4,12:d:- but express different H2-antigens. Serovars 4,12:d:- and Derby (4,12:f,g:[1,2]) shared the highest number of identical virulence determinants (Fig. 11). Only 7 of the 104 markers determined differed between both serovars. Four genes *pagK, rhuM*, *sugR* and *lpfD* were present additionally, whereas *sopD2*, STM4595, and *stjB* were absent. Serovars Paratyphi B dT+ and Duisburg showed the second highest similarity. Nine of the 104 virulence determinants were different.

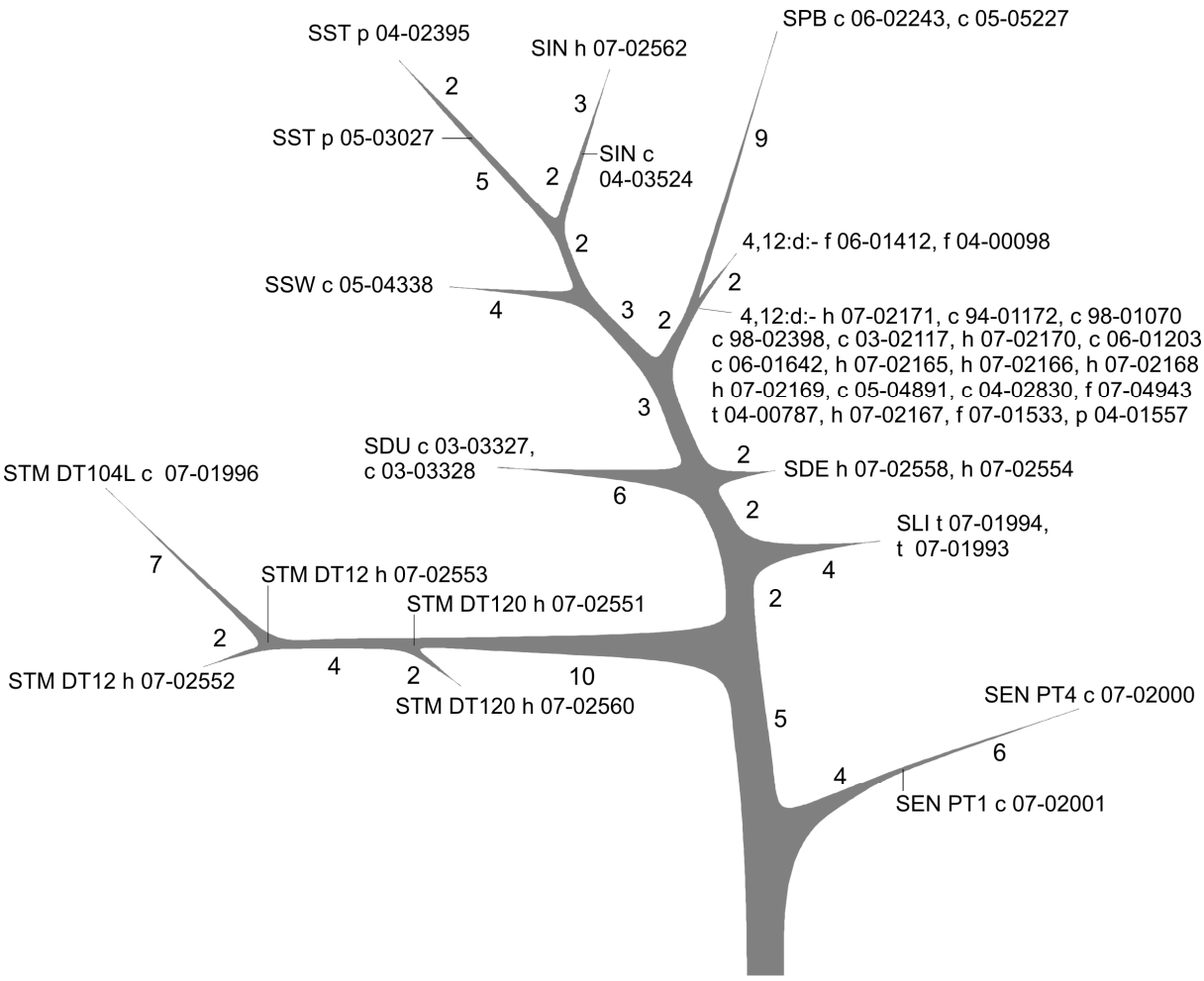


Figure 11: Rendered maximum parsimony tree. The tree shows the differences between the *Salmonella* isolates based upon 104 virulence determinants. The count indicates the number of genes different between the branches. Strain information is given in the order serovar, isolation origin, and strain number. Abbreviations: SSW, serovar Schwarzengrund; SST, serovar Stanley; SDU, serovar Duisburg; SEN, serovar Enteritidis; STM, serovar Typhimurium; SIN, serovar Infantis; SLI, serovar Livingstone; SDE, serovar Derby; SPB dT+, serovar Paratyphi B dT+; h, human; t, turkey; c, chicken; p, pig; f, feed.

The two serovar Livingstone strains shared with serovar 4,12:d:- as unique serovar the same set of fimbrial genes, including an additional *lpfD*. The serovars Stanley and Schwarzengrund harboured other sets of fimbrial markers. Serovar Stanley carried in addition the genes *lpfD*, *stcC*, and *tcfA*, and serovar Schwarzengrund lacked the markers *steB*, *stfE* and *stjB* but carried *tcfA*. The two serovar Derby strains and the two serovar Infantis strains lacked the markers *stjB* and STM4595. Both serovars also carried *lpfD* and serovar Infantis furthermore *tcfA*. Serovars Enteritidis and serovar Typhimurium encoded more fimbrial clusters than serovar 4,12:d:-. The two serovar Enteritidis strains carried additionally the *lpfD*, *pefA*, Prot6E, *sefA*, and *sefR* fimbrial genes but lacked *stjB*. All five serovar Typhimurium strains carried in addition *lpfD* and *stcC* and lacked *steB*. Moreover one serovar Typhimurium strain carried *pefA*.

The recently described *Salmonella* Genomic Island-1 in various serovars (30) could be only found in serovar Typhimurium phage type DT104 strains. However, *trhH* and the *rep* gene located in SGI-1 could be also found in other serovars/strains. The *trhH* gene was also present in serovars 4,12:d:- and Paratyphi B dT+, whereas the SGI-1 *rep* gene could be found in all serovars analysed.

3.2.4 Other characteristics of serovar 4,12:d:-

All 21 serovar 4,12:d:- strains were negative for genes conferring antimicrobial resistance. Only two strains were positive for the insertion-sequence common region 1 (ISCR1) element and three strains for the ISCR3 element. These ISCR elements are associated with the presence of antibiotic resistance (238).

In the microarray, 17 incompatibility group (Inc) marker probes according to Caratolli *et al*. (36) were present. Inc markers detect the presence of plasmids in strains. The most prevalent Inc group was Inc W, present in 7 of the 21 serovar 4,12:d:- strains. Moreover, one strain showed the presence of Inc FIA, and another strain showed Inc P. Twelve strains showed no Inc markers indicating the absence of any plasmids.

Three transposases of IS elements were always present, namely SPA2465, IS*200* and the IS*1351*-like transposase-encoding gene. A fourth transposase, STY343, was detected in 19 of the 21 isolates. Other transposase-encoding genes were found only sporadically or were completely absent (Table S2, supplementary material on CD).

In comparison to serovar Typhimurium, serovar 4,12:d:- strains lacked several metabolism genes, namely the genes *dgoA*, *hsdM*, *oafA*, STM1896 and STM4497. STM3782 was only in 15 out of 21 strains present. The *pflD* gene was present in all serovar 4,12:d:- strains isolated from human, whereas it was absent in all serovar 4,12:d:- strains isolated from other origins.

3.3 Investigation of *S. enterica* subsp. *enterica* serovar Paratyphi B dT+ and serovar 4,5,12:b:-

3.3.1 Antimicrobial resistance characteristics of serovar Paratyphi B dT+

Three out of the 36 serovar Paratyphi B dT+ strains were fully susceptible to the 17 antimicrobials tested. The other 33 serovar Paratyphi B dT+ strains exhibited phenotypically antimicrobial multi-drug resistance (Appendix I, Table A4). The resistance genotypes that were responsible for their resistance phenotypes were determined by microarray analysis and

confirmed by PCR (Table S2, supplementary material on CD). One exception occurred in one serovar Paratyphi B dT+ O:5 antigen negative (O:5-) strain encoding *sul2* without conferring phenotypic resistance to sulfamethoxazole. Figure 12 shows the analysed resistance determinants for each strain. Thirty of the strains carried a class 2 integrase gene and 14 strains a class 1 integrase gene. Coexisting phenotypic trimethoprim, spectinomycin and streptomycin resistance and the detection of *dfrA1*, *sat1(*Tn*7)* and *aadA1* in a strain indicated that a class 2 integron harbouring these resistance gene cassettes is carried by the strains as previously described by Miko *et al*. (165). This combination of targets could be found in 30 strains positive for *int2*, only one of which was O:5 antigen positive (O:5+) (07-01989). The co-existence of class 1 and class 2 integrases was found in 12 strains. Three strains possessed class 1 integrase alone. In three serovar Paratyphi B dT+ O:5- isolates (07-01767, 07-04825 and 08-00832) the extended-spectrum *ß*-lactamase $bla_{\text{CTX-M2}}$ gene could be detected. In another serovar Paratyphi B dT+ O:5- isolate (07-01758), a different extended-spectrum *ß*-lactamase bla_{TEM52} gene (according to accession no. EF141186) could be detected (data not shown). These strains were resistant to ceftiofur and amoxicillin/clavulanic acid.

Three O:5+ group strains (07-01653, 07-01985, and 07-01986) harboured the *Salmonella* Genomic Island-1 as described for *Salmonella enterica* serovar Typhimurium DT104 (30) indicated by the genes *int*_SGI1 *(S001)*, *rep*_SGI1 *(S003)*, *trhH (S012)*, *res*_SGI1 *(S027)*, *int1 (S028)*, *aadA2 (S029)*, *qacEΔ1 (S030)*, *floR (S032)*, *tet(*G*) (S034)*, bla_{PSE1} *(S038)*, *sul1 (S040)*, and *tnpA*_IS6100 *(S043)*.

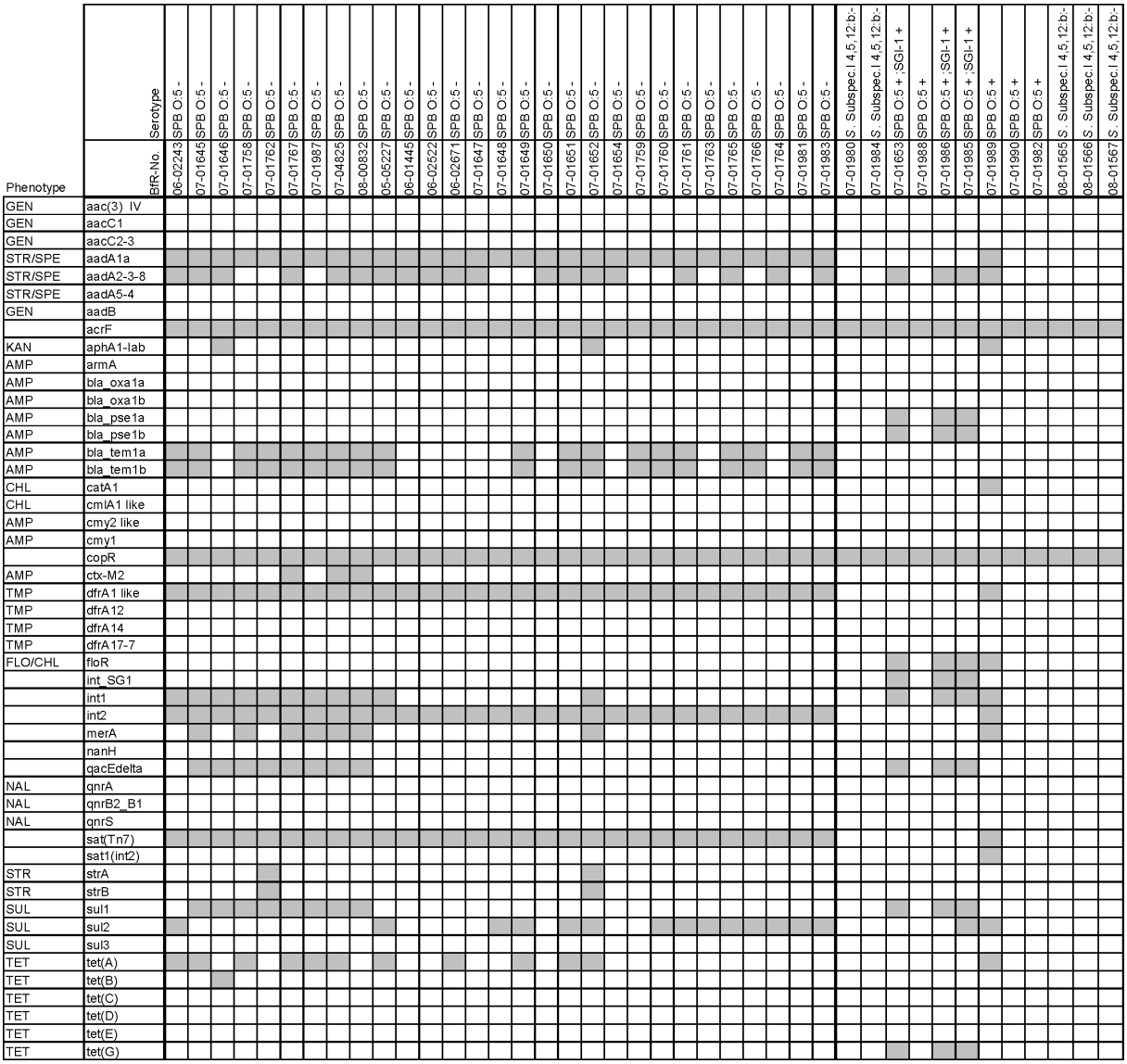
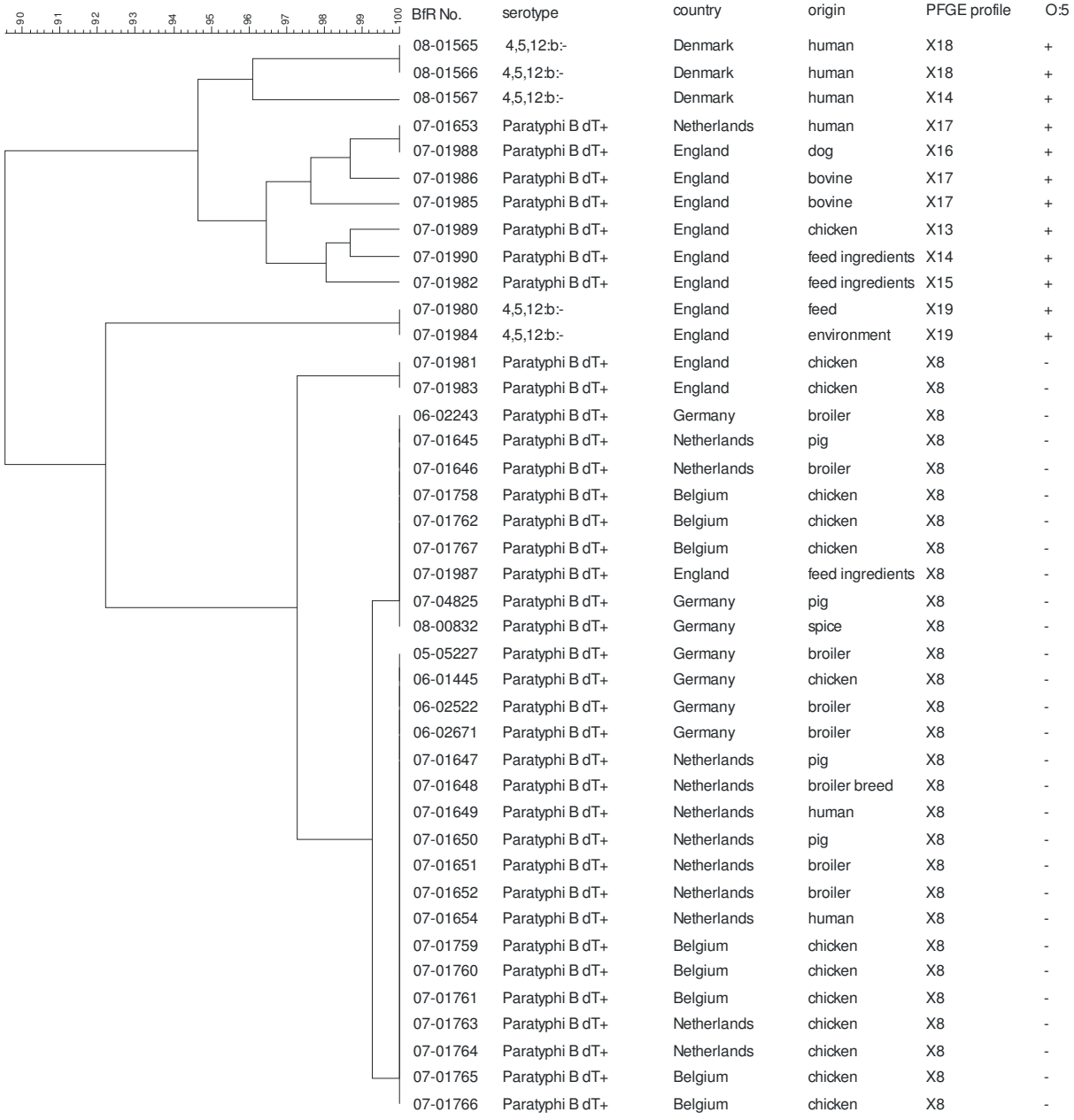


Figure 12: Resistance determinants microarray data of the 41 strains analysed. On top the strain numbers and the corresponding serovar (SPB = serovar Paratyphi B dT+) or serovar 4,5,12:b:- are indicated. Strains harbouring the O:5 antigen are indicated by addition of O:5+. On the left side the analysed genes are listed in alphabetical order resulting in resistance to several antibiotics showed in abbreviations or genetic resistance elements like integron associated integrases and transferases. One column within the figure shows the hybridisation result of a particular strain. A grey box indicates the presence, a white box the absence of the target sequence in the strain.

Within 28 strains insertion-sequence common regions, ISCR1 (three), ISCR2 (twenty) and ISCR3 (eight) could be detected (data not shown). The three strains positive for ISCR1 also possessed an ISCR2 element. Insertion-sequence common regions are associated with the presence of antibiotic resistance (238). ISCR2 microarray signals corresponded to *sul2* signals.

3.3.2 Analysis of population structure

A dendrogram was constructed based on the presence or absence of 104 virulence determinants (83 pathogenicity and 21 fimbrial markers) of 36 serovar Paratyphi dT+ and 5 serovar 4,5,12:b:- strains (Fig. 13).



BfR No.	serotype	country	origin	PFGE profile	O:5
08-01565	4,5,12:b:-	Denmark	human	X18	+
08-01566	4,5,12:b:-	Denmark	human	X18	+
08-01567	4,5,12:b:-	Denmark	human	X14	+
07-01653	Paratyphi B dT+	Netherlands	human	X17	+
07-01988	Paratyphi B dT+	England	dog	X16	+
07-01986	Paratyphi B dT+	England	bovine	X17	+
07-01985	Paratyphi B dT+	England	bovine	X17	+
07-01989	Paratyphi B dT+	England	chicken	X13	+
07-01990	Paratyphi B dT+	England	feed ingredients	X14	+
07-01982	Paratyphi B dT+	England	feed ingredients	X15	+
07-01980	4,5,12:b:-	England	feed	X19	+
07-01984	4,5,12:b:-	England	environment	X19	+
07-01981	Paratyphi B dT+	England	chicken	X8	-
07-01983	Paratyphi B dT+	England	chicken	X8	-
06-02243	Paratyphi B dT+	Germany	broiler	X8	-
07-01645	Paratyphi B dT+	Netherlands	pig	X8	-
07-01646	Paratyphi B dT+	Netherlands	broiler	X8	-
07-01758	Paratyphi B dT+	Belgium	chicken	X8	-
07-01762	Paratyphi B dT+	Belgium	chicken	X8	-
07-01767	Paratyphi B dT+	Belgium	chicken	X8	-
07-01987	Paratyphi B dT+	England	feed ingredients	X8	-
07-04825	Paratyphi B dT+	Germany	pig	X8	-
08-00832	Paratyphi B dT+	Germany	spice	X8	-
05-05227	Paratyphi B dT+	Germany	broiler	X8	-
06-01445	Paratyphi B dT+	Germany	chicken	X8	-
06-02522	Paratyphi B dT+	Germany	broiler	X8	-
06-02671	Paratyphi B dT+	Germany	broiler	X8	-
07-01647	Paratyphi B dT+	Netherlands	pig	X8	-
07-01648	Paratyphi B dT+	Netherlands	broiler breed	X8	-
07-01649	Paratyphi B dT+	Netherlands	human	X8	-
07-01650	Paratyphi B dT+	Netherlands	pig	X8	-
07-01651	Paratyphi B dT+	Netherlands	broiler	X8	-
07-01652	Paratyphi B dT+	Netherlands	broiler	X8	-
07-01654	Paratyphi B dT+	Netherlands	human	X8	-
07-01759	Paratyphi B dT+	Belgium	chicken	X8	-
07-01760	Paratyphi B dT+	Belgium	chicken	X8	-
07-01761	Paratyphi B dT+	Belgium	chicken	X8	-
07-01763	Paratyphi B dT+	Netherlands	chicken	X8	-
07-01764	Paratyphi B dT+	Netherlands	chicken	X8	-
07-01765	Paratyphi B dT+	Belgium	chicken	X8	-
07-01766	Paratyphi B dT+	Belgium	chicken	X8	-

Figure 13: Numerical coefficient cluster analysis of pathogenicity and fimbrial genes. A Pearson correlation and unweighted pair group method with arithmetic mean dendrogram shows the relatedness between strains. The scale on top (left) shows similarities in percentages with respect to the number of concordant genes between the strains. Strain number, serotype, country, origin, the *Xba*I pulsed-field gel electrophoresis (PFGE) profile, and presence (+) or absence (-) of O:5 antigen is indicated right to the dendrogram. Three strains (07-01653, 07-01985, and 07-01986) harboured the *Salmonella* Genomic Island-1. dT+, (+)-tartrate fermenting.

The dendrogram shows two main clusters comprising distinct groupings of serovar Paratyphi dT+ O:5- and serovar Paratyphi dT+ O:5+ isolates. These microarray-based

population clusters could be confirmed by pulsed-field gel electrophoresis (PFGE). A comparison of *Xba*I PFGE profiles including serovar 4,5,12:b:- isolates subdivided the 36 serovar Paratyphi B dT+ and five serovar 4,5,12:b:- isolates again into two main groups. The first group was represented by all O:5- strains including isolates from chickens, pigs, and humans originating from the Netherlands, Belgium, and Germany as well as three O:5- British strains showing an identical X8 PFGE profile (Fig. 14, lane 2) characteristic for the poultry associated type previously described by Miko *et al.* (165). The second group was heterogeneous with respect to their PFGE profiles (Fig. 14, lane 3-9). It contained all O:5+ British strains and one O:5+ Dutch strain as well as all five monophasic O:5+ serovar 4,5,12:b:- strains. Two serovar 4,5,12:b:- strains from England (07-01980 and 07-01984) were grouped in their own branch located between the O:5+ and O:5- group (Fig. 13). However, these strains can be assigned to the O:5+ group since characteristic genes (*sopD*, *srfJ*, *lpfD*, *spvR*, *and stcC*) for this group were present in both serovar 4,5,12:b:- strains.

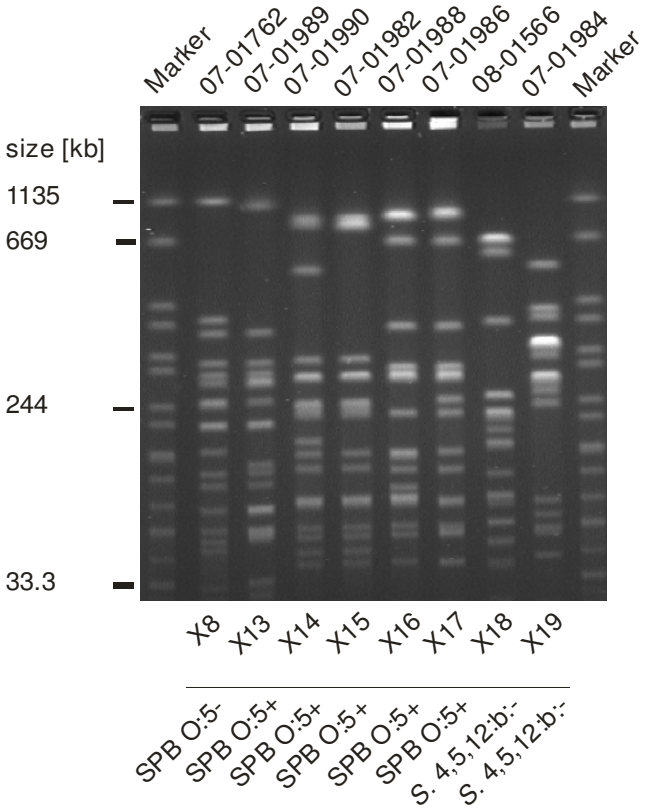


Figure 14: Pulsed-field gel electrophoresis (PFGE) profiles of representative *S. enterica* subsp. *enterica* strains after digestion with *Xba*I. As molecular weight standard (Marker) *Salmonella* Braenderup reference strain H9812 was used (Lanes 1 and 10). Lanes 2-9 represent the unique PFGE pattern of the poultry-associated serovar Paratyphi B dT+ (SPB) O:5- and seven different PFGE types of serovar SPB O:5+ as well as the monophasic serovar 4,5,12:b:-. Numbers are indicated above the gel strain.

3.3.3 Virulence determinant characterisation of serovar Paratyphi B dT+

Figure 15 shows the virulence and fimbrial gene set for each strain. All strains gave positive signals with the *Salmonella* Pathogenicity Islands (SPI) 2, 4 and 5 targets. Variations were observed in SPI-1 and SPI-3. Four serovar Paratyphi B dT+ O:5+ isolates from Britain and one Dutch isolate lacked the *avrA* gene located in SPI-1. AvrA stabilizes cell permeability and tight junctions in intestinal epithelial cells (135). SPI-3 showed a truncation in 18 (62%) strains of the O:5- isolates represented by the absence of *sugR*.

Moreover, one serovar 4,5,12:b:- strain (07-01984) lacked the *ruhM* located adjacent to *sugR*. All other strains belonging to the O:5- group as well as all strains of the O:5+ group, possessed a complete SPI-3. SPI-7, previously described in serovar Typhi and serovar Paratyphi A (180), could not be detected. The O:5- group lacked almost all virulence genes encoded by the prophages Fels-1, Gifsy-1, Gifsy-2 and Gifsy-3. However, *sodC1*, located in Gifsy-2 (encoding a superoxide dismutase) was present in two strains (07-01981 and 07-01983). Four serovar Paratyphi B dT+ O:5+ and two serovar 4,5,12:b:- strains possessed a complete set of Gifsy-2 encoded virulence genes.

The other four strains of the O:5+ group showed a truncated Gifsy-2 region. Only two serovar 4,5,12:b:- strains (07-01980 and 07-01984) of this group lacked the region completely. The *pagJ* gene located within Gifsy-3 was present in six out of seven serovar Paratyphi B dT+ O:5+ and three serovar 4,5,12:b:- strains. Fels-2 was present in 85% of the O:5+ group strains but mostly truncated downstream (data not shown). Other recognised prophage-associated virulence determinants were *irsA* (38 out of 41 strains), encoding an internal response element to stress and *sodC1* in the three Danish serovar 4,5,12:b:- isolates encoding a super oxide dismutase. All strains showed the presence of the *sopE2* gene which is highly homologous to *sopE1*, encoding another secreted outer protein (13). The *sopE2* is broadly distributed in *Salmonella* suggesting that conservation of this gene may be more important than of *sopE1*.

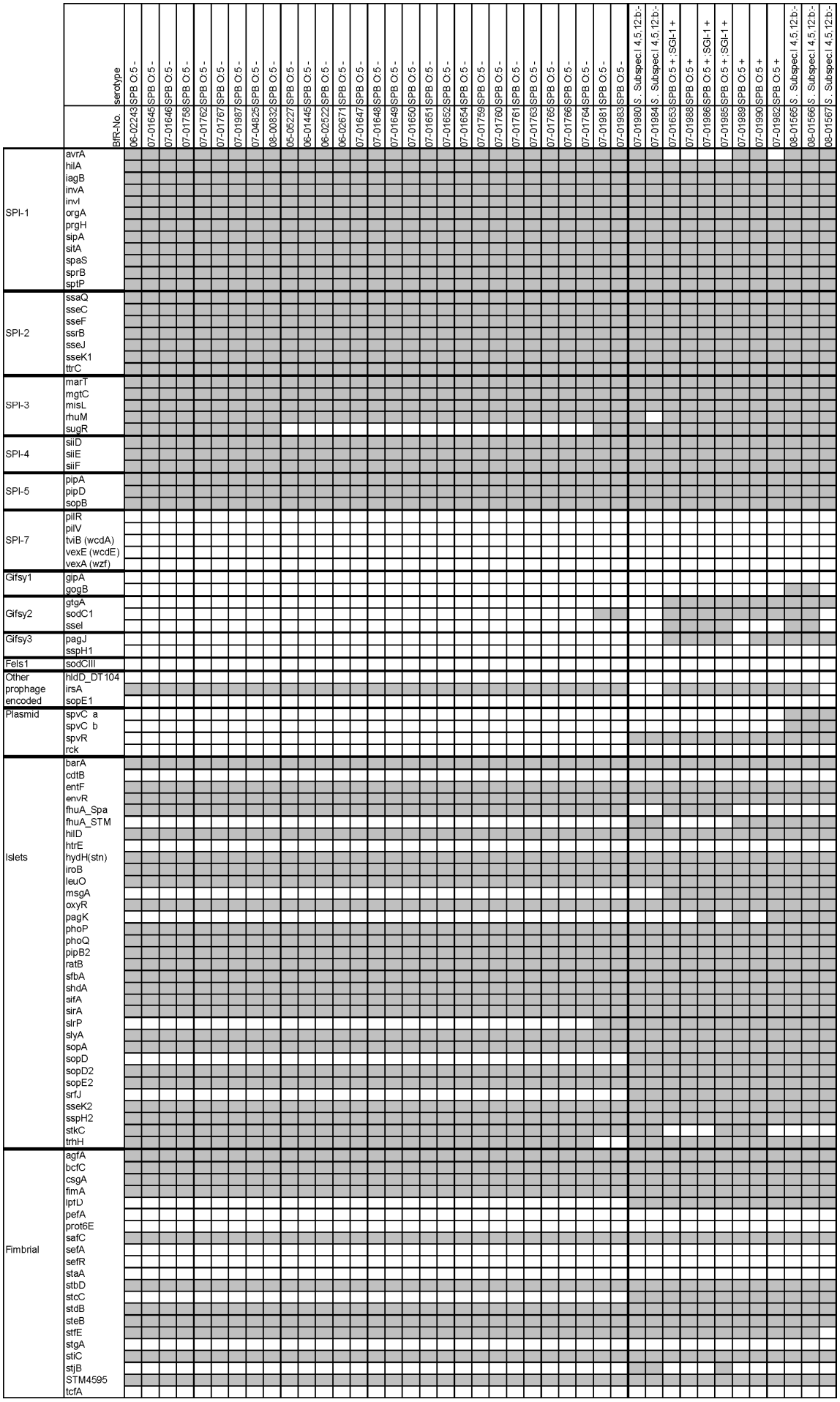

BfR-No.
serotype
06-02243|SPB O:5 -
07-01645|SPB O:5 -
07-01646|SPB O:5 -
07-01758|SPB O:5 -
07-01762|SPB O:5 -
07-01767|SPB O:5 -
07-01987|SPB O:5 -
07-04825|SPB O:5 -
08-00832|SPB O:5 -
05-05227|SPB O:5 -
06-01445|SPB O:5 -
06-02522|SPB O:5 -
06-02671|SPB O:5 -
07-01647|SPB O:5 -
07-01648|SPB O:5 -
07-01649|SPB O:5 -
07-01650|SPB O:5 -
07-01651|SPB O:5 -
07-01652|SPB O:5 -
07-01654|SPB O:5 -
07-01759|SPB O:5 -
07-01760|SPB O:5 -
07-01761|SPB O:5 -
07-01763|SPB O:5 -
07-01765|SPB O:5 -
07-01766|SPB O:5 -
07-01764|SPB O:5 -
07-01981|SPB O:5 -
07-01983|SPB O:5 -
07-01980|S. Subspec I 4,5,12:b:-
07-01984|S. Subspec I 4,5,12:b:-
07-01653|SPB O:5 + ,SGI-1 +
07-01988|SPB O:5 +
07-01986|SPB O:5 + ,SGI-1 +
07-01985|SPB O:5 + ,SGI-1 +
07-01989|SPB O:5 +
07-01990|SPB O:5 +
07-01982|SPB O:5 +
08-01565|S. Subspec I 4,5,12:b:-
08-01566|S. Subspec I 4,5,12:b:-
08-01567|S. Subspec I 4,5,12:b:-
SPI-1
avrA
hilA
iagB
invA
invI
orgA
prgH
sipA
sitA
spaS
sprB
sptP
SPI-2
ssaQ
sseC
sseF
ssrB
sseJ
sseK1
ttrC
SPI-3
marT
mgtC
misL
rhuM
sugR
SPI-4
siiD
siiE
siiF
SPI-5
pipA
pipD
sopB
SPI-7
pilR
pilV
tviB (wcdA)
vexE (wcdE)
vexA (wzf)
Gifsy1
gipA
gogB
Gifsy2
gtgA
sodC1
sseI
Gifsy3
pagJ
sspH1
Fels1
sodCIII
Other prophage encoded
hldD_DT104
irsA
sopE1
Plasmid
spvC a
spvC b
spvR
rck
Islets
barA
cdtB
entF
envR
fhuA_Spa
fhuA_STM
hilD
htrE
hydH(stn)
iroB
leuO
msgA
oxyR
pagK
phoP
phoQ
pipB2
ratB
sfbA
shdA
sifA
sirA
slrP
slyA
sopA
sopD
sopD2
sopE2
srfJ
sseK2
sspH2
stkC
trhH
Fimbrial
agfA
bcfC
csgA
fimA
lptD
pefA
prot6E
safC
sefA
sefR
staA
stbD
stcC
stdB
steB
stfE
stgA
stiC
stjB
STM4595
tcfA

Figure 15: Virulence determinants microarray data of the 41 strains analysed. On top the strain numbers and the corresponding serovar [SPB, *Salmonella* Paratyphi B (+)-tartrate fermenting] or *S*. 4,5,12:b:- are indicated. Strains harbouring the O:5 antigen are indicated by addition of O:5+. On the left side, the analysed genes are grouped according to their particular genomic location (*Salmonella* Pathogenicity Islands SPI-1 to SPI-7, Prophages Gifsy-1, Gifsy-2, Gifsy-3, Fels-1, plasmids, islets) or function (fimbrial). The column below the strain designation shows the hybridisation result of a particular strain. A grey box indicates the presence of the target sequence in the strain; a white box the absence.

Virulence determinants targets for *gogB, gtgA, sseI, pagJ, pagK, sopD, sopE1, spvC, spvR, fhuA*_STM*, msgA, srfJ* and the fimbrial associated genes *lpfD*, *stcC*, and *stjB* were exclusively present in O:5+ group strains (Table 11). The virulence gene repertoire of three SGI-1 positive strains was highly similar (Fig. 15). One strain (07-01986) was additionally positive for *pagK*.

Table 11: Pathogenicity and fimbrial genes exclusively present in 12 *Salmonella enterica* serovar Paratyphi B dT+ O:5+ strains

Gene name	**% Presence in O:5+ group strains (No. of strains)**	**Function**
gogB	17 (2)	Gifsy-1 encoded leucine-rich type III secreted repeat protein
gtgA	83 (10)	Gifsy-2 encoded prophage protein
sseI	50 (6)	Gifsy-2 encoded prophage putative type III secreted protein
pagJ	67 (8)	Gifsy-3 encoded PhoP/PhoQ-activated protein
sopE1	25 (4)	P2-like cryptic bacteriophage encoded translocated effector
spvC	25 (4)	*Salmonella* plasmid virulence: hydrophilic protein
spvR	100	*Salmonella* plasmid virulence: regulation of spv operon, lysR
*fhuA*_STM	67 (8)	Outer membrane ferric hydroxamate receptor
msgA	83 (10)	SsrB-regulated factor encoding for macrophage survival
pagK	50 (6)	PhoP/PhoQ-activated protein
sopD	100	Type III secreted invasion protein
srfJ	100	Putative virulence factor, activated by transcription factor SsrB
lpfD	100	Long polar fimbrial operon protein
stcC	100	Putative outer membrane protein
stjB	25 (4)	Putative fimbrial usher protein

All serovar Paratyphi B dT+ O:5- strains lacked the typical *Salmonella* virulence plasmid represented by the absence of *spvC*, *spvR*, *rck* and *pefA* (157). All strains of the O:5+ group possessed the *spvR* (regulator for *spv* operon) gene but completely lacked also *rck* (encoding for resistance to complement killing) and *pefA* (plasmid encoded fimbriae). Three serovar 4,5,12:b:- strains were exclusively positive for *spvC* and harboured a variation of the *Salmonella* virulence plasmid indicated by the presence of and *spvR*.

3.3.4 Other characteristics of serovar Paratyphi B dT+

Several incompatibility group markers (Inc) according to Carattoli *et al*. (36) could be found indicating the presence of various plasmids in several strains. The most prevalent groups were Inc1 and IncK simultaneously present in 25 of the 41 serovar Paratyphi B dT+ and serovar 4,5,12:b:- strains. Ten strains showed the presence of IncHI2 which was always present together with Inc1/IncK. Additionally, the incompatibility group markers IncP and IncW have been sporadically found together with other frequently found Inc groups. Another strain (07-01646) possessed only the groups IncHI1 and IncFIA. All together six strains were negative for the probes representing the incompatibility groups tested (36) this indicates the absence of any plasmids. Transposases of the IS*200* element and SPA2465 were present in all strains, whereas IS*1351*-like (38 out of 41), STY343 (37 strains), IS*1*-like (27 strains), IS*6100* (23 strains) and of the IS-element Tn*3* (20 strains) occurred additionally. IS*26* and *Tn*2680 occurred together in 10 strains (data not shown). All strains possessed a *copR* gene encoding for copper resistance located on SPI-5 and an *acrF* gene encoding acriflavine resistance. In eight serovar Paratyphi B dT+ O:5- strains the *qacEΔ1 gene* (encoding quaternary ammonium resistance) and in seven strains *merA* (encoding for mercury resistance) were present. Both genes are associated with the class 1 integron structure.

The O5+ group showed exclusively presence of *oafA* encoding the O-antigen acetylase.

The metabolic gene *dgoA* encoding for a galactonate dehydrogenase was present in all serovar Paratyphi B dT+ and 4,5,12:b:- strains. STM3782 encoding for a galactitol specific enzyme was present in all except two serovar 4,5,12:b:- strains (data not shown). Other metabolic genes (data not shown) *hsdM* and *pflD* were present in almost all O:5+ strains (ten and twelve out of twelve) whereas they were almost lacking in O:5- strains (two and three out of 29). STM1896 and STM4497 encoding for two cytoplasm proteins that were mostly encoded by O:5- strains (28 out of 29 each) but not by O:5+ strains (seven out of twelve).

4. Discussion

Epidemiological analysis of food-borne pathogens is an important contribution to the understanding of clonality, dissemination, and the evolution of particular subtypes in animals, food and humans. In the case of *Salmonella,* 2579 serovars are currently known but only a few of them play a major role in the infection of humans. Molecular approaches aim to assess the genetic relatedness of isolates and within the last thirty years a number of molecular methods have been developed to type and characterise isolates (74). Since its initial description in 1983 (74,215) pulsed-field gel electrophoresis has been established as the preeminent "gold standard" molecular method to investigate the epidemiology of most bacterial pathogens. In PFGE, whole genomic DNA is cleaved by rare cutting enzymes and separated by the size of the restricted DNA fragments in an agarose gel. Although this approach is useful it does not reveal data on the gene repertoire and biological properties of a strain. For the identification of certain genes or gene sequences in a genome, PCR became an easy and cheap method. Owing to its commercialisation and standardisation lasting recent years, it is widely applied in diagnostic laboratories. Alternatively, hybridisation between complement single nucleic acid strands is another prominent method to detect specific sequences in DNA or RNA (225). This principle was used and advanced as a high-throughput system of many molecular properties simultaneously in one experiment, generally known as microarray technology (101). DNA microarrays enable the simultaneous identification of a set of hundreds of genes in a reasonable amount of time. In this study, the microarray technique has been applied for the genotypic characterisation of two epidemiological important *Salmonella enterica* serovars, frequently isolated from poultry.

For that purpose, a set of 276 57-60mer and 5 40-45mer oligonucleotide probes mirroring the genomic variability in *Salmonella enterica* subsp. *enterica* were designed. The probes were printed on glass slides to identify genes or defined sequence variation associated with the pathogenicity, resistance, fimbriae, prophages, mobility, metabolism and serotyping of salmonellae. The procedure consists of fluorescent labelling of whole genomic *Salmonella* DNA of the test strain followed by an 18 h hybridisation of this DNA with the oligonucleotide probes which were printed on glass slides in a defined order. Afterwards, the slide was scanned and raw data was further analysed. The outcome was a profile of fluorescent signals which indicate the presence or absence of the gene or gene sequence for the strains tested. The *Salmonella* DNA has been extensively validated 'in-house' so as to use it as a reliable tool for

routine characterisation at the *Salmonella* National Reference Laboratory (Berlin, Germany) in the future.

4.1 Development of the *Salmonella* DNA microarray

4.1.1 Development of the Salmonella *DNA microarray*

A couple of important characteristics were considered during microarray development. The slide surface, the oligonucleotide probe length and specificity as well as the fluorophore used for labelling and time of hybridisation are the most crucial points. For probe design the Array Designer software (Premier Biosoft) was used. It was decided to use oligonucleotides as probes instead of longer PCR products as published previously for some methods (118). PCR products often do not have enough discriminatory power to identify differences in short variable regions within a gene family, such as the *fliC* and *fljB* gene encoding the structural unit of *Salmonella* flagellae. The ability to differentiate was important for the characterisation and typing of *Salmonella* isolates in the approach described here. In addition, the production of oligo-based microarrays requires fewer resources than PCR product-based microarrays. The probe length of the single-stranded DNA oligonucleotides was set to 57-62 to obtain a high, robust and reproducible signal compared to short oligonucleotide probes for detecting single-nucleotide polymorphisms. (118). Sequences were obtained from the public database Genbank (release 2005; http://www.ncbi.nlm.nih.gov/). The number of designed probes was limited to a maximum of 300 for various reasons. First, the amount of information generated should be manageable within a reasonable analysis time. Second, the intention was to develop an array that is as robust as possible and suitable for regular use in the National Reference Laboratory. Therefore an extensive and rigid validation procedure was performed (see section 4.1.3). Such a validation can be done only with a reasonable number of probes. Third, the number of markers analysed correlates with the cost of the array and the cost of an analysis should be kept as low as possible. In order to minimize the number of optional targets, only prominent proxies have been selected. For example, fimbrial clusters are encoded by up to ten consecutive genes but only one representative gene was selected for probe design. Similarly, representative genes were selected from the five *Salmonella* Pathogenicity Islands scattered over the complete region in order to identify potential deleted regions. In summary, for all marker groups an adapted quantity of markers has been chosen.

Under optimal conditions the probe sequence was selected near the centre of the open reading frame of the gene. For validation, PCR primers were designed enclosing the probe sequence and resulting in a PCR product of approximately 400 bp size.

The availability of the probe sequence on the slide for the genomic DNA to be hybridised is a crucial step during hybridisation. Therefore, a number of preliminary experiments were performed with respect to the surface chemistry of the slides. Aldehyde- or epoxy coated slides were tested in terms of their optimal print properties, recognition (sensitivity) of probes and interaction (specificity) of the probes with the target DNA (data not shown). Finally, a n-succinimidyl, proprietary coated 3d-surface slide type (CodeLink activated slide, Amersham) was chosen enhancing the amount of bound probes by a hydrophilic polymer harbouring amine-reactive groups. This covalently cross linked polymer orients the fixed probes towards the surface of the slide. This orientation supports hybridisation because a higher percentage of probes are readily available for the DNA. The need for spacers like poly(dT) or poly-ethylene glycol (PEG) is eliminated by this surface. Moreover, passivity of the reactivity of immobilized DNA is supported by the hydrophilic ambience. The effect is a substantially lower background and therefore a broader signal gap between background and probe signal intensities. If compared with other slide materials, glass slides possess several advantages. First, in contrast to other materials coated glass surfaces enable the covalent attachment of nucleic acids. Second, the material is itself very durable and tolerates incubation with buffers of high ionic strength. Third, the surface is non-porous and therefore supports minimal volumes, which improves the binding kinetics of the targets. Finally, the low self fluorescence of the material generates a good signal to noise ratio. The printing was performed on and optimized for the Genetix QArray Mini printer (Genetix) using eight 75 µm tungsten split pins (Amersham). As printing buffer a sodium phosphate buffer in a concentration of 300 mM without any additives was used. SDS is often added in printing buffers. SDS, along with other tensides or organic solvents such as ethanol, affects the surface tension of the printed solution which has a strong influence on produced spot sizes (39). The absence of tensides in the printing buffer results in lower amounts of solved probes but supports the printing of smaller and uniform spots. It was observed that spot diameters in sodium phosphate buffer without the addition of SDS resulted in stronger signal intensities compared to buffer with the addition of SDS (data not shown).

The oligonucleotide probes designed in this study were printed on the CodeLink activated glass slides in two array fields each of 768 spots (see Fig. 5). The fluorophore dyes AlexaFluor555 or AlexaFluor647 were used for the labelling of genomic *Salmonella* DNA

because of their better ability to withstand bleaching trough UV light and their robust handling abilities whilst undergoing staining and hybridisation. In contrast to the alternative dyes Cy5 and Cy3 which are often used for labelling in microarray experiments, these next generation fluorophores are significantly more stable and enabled the rescan of slides after a couple of weeks.

The hybridisation and washing procedure was optimized (data not shown) for the DNA microarray developed here. The success of the hybridisation depends highly on the amount and purity of the DNA. Our experiments showed that for labelling followed by hybridisation, at least 4 µg of pure genomic *Salmonella* DNA should be used for reliable and high quality signals (data not shown). If the DNA is contaminated by cell debris as a result of the cell lyses process, the fluorophores can bind to them possibly leading to a lack of sufficient fluorphores for the DNA labelling reaction. Furthermore, the unspecific binding of fluorophores to cell debris generates a higher signal-background noise because of the random sticking of these particles to probes. Second, it blocks residual binding sites for the probes which lower the target signals.

After preliminary studies the hybridisation time was set to 18 hours producing the highest signal to background noise. After 18 hours the target to probe hybridisation equilibrium has obviously been reached. However, Sartor *et al.* (210) recommends different and much longer times. Thus, the equilibrium heavily depends on the concentration and melting temperature of the target and needs to be optimized for the application used.

After hybridisation the remaining hybridisation mixture was washed from the slides. Glass slides enable the use of washing buffers with high ionic strength. In this study, a SDS containing washing buffer was used followed by a SDS free washing buffer and subsequently rinsing by double-distilled water. After washing, the DNA microarray slides were centrifuged for drying and scanned for hybridised, labelled DNA. The scanner uses two laser beams which are refracted by a dual beam-splitter forming a focused beam which stimulates the fluorescence of incorporated dye molecules. The fluorescence of labelled DNA was simultaneously in the Cy3 and Cy5 channel measured. The cross talk between the two channels can be minimized by subsequent scanning of the channels. However, in the developed approach no differences were measured between subsequent or simultaneous scanning (data not shown). Being twice as fast, the simultaneous scan was preferred.

Occasionally after scanning, on the slides large “green coloured clouds” have been observed leading to higher signal to background noise. These “clouds” probably appeared

because of the incomplete washing of ingredients derived from the blocking buffer. Residues of the blocking buffer could be removed by an additional washing step with double-distilled water and upright placed slides during centrifugation for drying.

4.1.2 Internal hybridisation and print control (IHC)

A number of controls have been included on the array. The positive (*Salmonella* target specific probe) and negative (*Salmonella* non-target specific probes) are important to indicate any deviations of the standardised performed hybridisation procedure. Furthermore, a contamination control (spotting buffer) indicates that microarray pins have been correctly washed between the exchanges of each probe. An additional control has been developed which has the aim of controlling the presence of each spot. This control is called internal hybridisation control (IHC). It functions similarly to an internal competitive amplification control described for diagnostic PCR (204). The control is simultaneously hybridised with the *Salmonella* genomic test-DNA to each probe spotted on the glass slide. If the test-DNA was negative for the probe, the internal control probe has to be positive indicating the presence of the probe (spot) on the slide and indicating the correct hybridisation conditions. If the *Salmonella* test-DNA and control DNA are negative the experiment has to be repeated because print or hybridisation failures occurred. The IHC consists of a mixture of all probes printed on the array. To ensure the sufficient annealing of complementary sequences of the test-DNA the concentration of the IHC needs to be titrated. Each probe of the mixture uniquely bound itself to the probe spotted. The IHC improves the accuracy of the microarray data and has not been described before for this type of DNA microarray. Moreover, it reliably detected un-printed oligonucleotides by the absence of a signal in the Cy5-channel. Owing to competitive hybridisation between IHC and genomic test-DNA the intensity of signals was lowered approximately 25% in average in comparison with hybridisation without IHC (data not shown). This is however a reasonable loss of intensity because of the higher quality of signals received.

4.1.3 Validation of the DNA microarray

In order to test the validity of the developed microarray, 23 *Salmonella enterica* subsp. *enterica* serovar reference strains and one *E. coli* reference strain were hybridised to the microarray and the results of the signals for each probe were compared with the results obtained by PCR for the corresponding probe. Altogether, 256 target primers, excluding some serotyping markers, were constructed or selected from literature and used for PCR screening

to identify the absence or presence of the target sequences among the 23 *Salmonella* reference strains and one *E. coli* reference strain (Table S1, supplementary material on CD). Probes of the serotyping marker group were confirmed by classical serotyping according to the White-Kaufmann-Le Minor scheme (186). To classify a microarray signal as present or absent a normalisation of probe intensities was evaluated using an integrated positive control probe for those sequences thought to be present in all *Salmonella* strains. The *ttrC* gene was selected as a target for the positive control probe because it is highly conserved in *Salmonella* strains (153). A ratio was calculated based upon the intensity of the specific probe by the mean intensity of both *ttrC* probes present on each block. A ratio of ≤0.15 indicated the absence; whereas a ratio of ≥0.25 normalised units indicated the presence of the specific gene sequence. Values between 0.15 and 0.25 normalised units were classified as uncertain. The threshold level validity of microarray signals was deviated by the comparison of the signals with the presence or absence of the gene by PCR taking into account all 23 *Salmonella* reference strains. This approach has been published previously using 35-40mers oligonucleotide probes (152). A new oligonucleotide probe sequence was designed for probes with results indicating continuously uncertain intensities or positive PCR results but negative microarray results.

The comparison between microarray and PCR signals gave an agreement of 94.9% (6114 data signals). Of these 41.7% (2687 data signals) were positive and 53.2% (3427 data signals) were negative signals. 3.5% (226 data signals) gave inconsistent results between PCR and microarray, 1.6% (100 microarray data signals) were classified as uncertain (see Table 9). The differences between the inconsistent results were primarily caused by probes linked to mobile elements 27.8% (63 data signals), e.g. ISCR1, iteronsP, rep_SG1, rep_DT193, repC_R64, repW, tnp_IS1202, tnp_IS1696, tnpA_ISlike. In cases of the probes for the genes *pefA*, *rhuM* and *sugR* a higher rate of wrong negative results or intensities within the uncertain range occurred. These probes were redesigned and the new probes were validated again by PCR. However, in some cases cross-hybridisations were unavoidable due to a highly similar sequence between two genes. For example, the probes detecting *pagJ*/*pagK* could not be redesigned. The comparison showed 90% sequence similarity on the DNA level and 83% on the predicted protein sequence (93). However, generally, the comparison between PCR and microarray data showed that 60% (139 signals) were identified as wrong negative PCR results. The reason might be that because shorter sequences in comparison to the probe sequences the PCR primers are more sensitive to single nuclear polymorphisms resulting in negative PCR.

It has been observed that in some reference strains a probe signal was lower than in other reference strains but still classified as positive. This is probably caused by single nuclear polymorphism in the target sequence. Experiments based on various nucleotide exchanges of the control probe ttrC showed that the sequence can differ up to 20% with the complementary *Salmonella* target DNA in order to generate a spot intensity after hybridisation which is classified as present (ratio ≥0.25). This observation was confirmed by others using 50mer oligonucleotide probes showing a similar decrease of signal intensity caused by single nuclear polymorphisms (118). Probes often gave positive signals, whereas the corresponding PCR products were negative. Apparently the probes derived from such targets might give cross signals with other homologous targets within the genome. Alternatively, PCR primers did not result in an expected PCR product, because of a truncated gene. It might also be possible that polymorphic sites occur in the target sequence of the PCR primer leading to weak or no PCR products. In contrast, 60mer oligonucleotide probes can tolerate a number of single nuclear polymorphism in the probe sequence as mentioned above. In conclusion, the developed and validated *Salmonella* microarray is a valuable tool to characterise genotypically isolates of serovars belonging to *Salmonella enterica* subsp. *enterica*. With its rigid validation process the robustness of the microarray is well documented. By the inclusion of an internal hybridisation control the reliability of the results has been significantly increased and makes the array potentially useful not only as a research tool but also for diagnostic purposes.

4.2 Application of the DNA microarray to poultry associated *Salmonella* serovars

In Germany the serovars 4,12:d:- and Paratyphi B dT+ are highly prevalent in poultry and poultry products. In contrast to serovar 4,12:d:-, serovar Paratyphi B dT+ possess chromosomally encoded resistances to several antimicrobials (164). Up to now few data about virulence determinants of both serovars, 4,12:d:- and Paratyphi B dT+ has been published. The aim was to determine clonality, virulence and resistance gene repertoire for both serovars in two studies. PFGE and the *Salmonella* DNA microarray (developed in this thesis) were selected as molecular analysis tools for both serovars.

4.2.1 Application of the Salmonella *DNA microarray to study the gene repertoire in* S. enterica *serovar 4,12:d:-*

With a serovar prevalence of 23.6% *Salmonella enterica* subsp. *enterica* serovar 4,12:d:- was the most frequently encountered serovar in German broiler flocks in 2005 to 2006 (65). In Denmark and the United Kingdom its prevalence was 15.15% and 2.8% respectively.

Obviously this emerging pathogen is well established in the production lines. However in contrast, this serovar currently has a low infection rate in humans. In order to elucidate this contradicting situation, because broilers are known as a major source of human salmonellosis, representative isolates of this serovar were characterised on the molecular level in order to describe their repertoire of virulence and resistance determinants, the relationship to other poultry associated serovars and to estimate its risk potential for humans. As molecular analysis tools PFGE and the new DNA microarray developed in this thesis were selected. Isolates of serovar 4,12:d:- from feed, animals and humans were investigated. Furthermore, for the comparison of the genetic relationship, other poultry associated serovars and serovars which had the same somatic (O) antigen and phase-1 flagellar (H1) antigen as serovar 4,12:d:- but expressed in addition a phase-2 (H2) flagellar antigen, were included (Appendix I, Table A3). PFGE and microarray data presented in this study showed that serovar 4,12:d:- possesses a highly clonal structure which spread successfully in poultry and can sporadically cause salmonellosis in humans. *S. enterica* serovar Paratyphi B dT+ which was the second prevalent serovar in German broilers in 2005 to 2006 (65) has also been previously described as clonal serovar (164). However, in contrast to the multi-drug resistant serovar Paratyphi B dT+ clone the 56 serovar 4,12:d:- isolates investigated in this study were shown to be completely susceptible to antimicrobial agents by phenotypic and genotypic methods. This is difficult to understand, because resistance determinants can easily spread by horizontal gene transfer (189). Consequently, cohabitating serovars from poultry which are under similar selective pressure caused by, e.g. the use of antibiotics for poultry production, could disseminate resistance determinants leading to similar, if not identical, phenotypes. This observation has to be elucidated in the future. Possibly, either a genetic barrier hampers the acquisition of resistance determinants into the serovar 4,12:d:- genome, or the flocks infected with this serovar are not under selective pressure because of the prudent use guidelines for minimal use of antimicrobial substances. Unfortunately, no correlation between the prevalence of multi-drug resistant serovar Paratyphi B dT+ and serovar 4,12:d:- with respect to antimicrobial usage can be provided.

The data also indicates that serovar 4,12:d:- evolved as a discrete serovar and has not emerged solely by deletion of the phase-2 flagellar antigen-encoding region of a biphasic serovar. Various genetic differences of serovar 4,12:d:- to serovars which had the same somatic (O) antigen and phase-1 flagellar (H1) antigen as serovar 4,12:d:- but express in addition a phase-2 (H2) flagellar antigen could be observed, especially virulence markers encoded by prophages (Fig. 10). The genetic non-relatedness is also supported by the finding

that the SPI-3 3'-region of serovar 4,12:d:- is truncated compared to those of the other serovars. In all serovar 4,12:d:- isolates analysed, the genes *sugR* and *rhuM* were absent. Amavisit *et al*. (5) have described several variations in SPI-3 in different serovars. They concluded that the acquisition of the *sugR-rhuM* region is likely a relatively recent event. The virulence gene content of serovar 4,12:d:- was most similar to that of serovar Derby (seven virulence determinants different) although it is rarely isolated from poultry. No similarity between serovars Derby and 4,12:d:- could be recognised in the PFGE profile (Fig. 9). Consequently, these data do not support any close relationship to serovar Derby. In further studies the number of strains and serovars compared for their gene repertoire to the serovar 4,12:d:- repertoire will be expanded to confirm these initial results.

The virulence gene repertoire of serovar 4,12:d:- showed that all five *Salmonella* Pathogenicity Islands were present. The most striking result was the complete absence of any virulence determinants encoded by prophages and an absence of plasmids in the majority of the strains. It has been proven that a virulence plasmid bearing the operon *spv* can be necessary to cause severe systemic disease (136). In addition, the *lpf* fimbrial operon encoding the long polar fimbriae was absent in all strains. It was shown that long polar fimbriae of serovar Typhimurium mediates adhesion to murine Peyer's patches and are required for full virulence (20). Fimbriae are responsible for the initial adhesion of the bacterium to the eukaryotic cells. They are frequently highly host specific and therefore an obvious factor that potentially influences host range. The absence of fimbrial clusters in serovar 4,12:d:- might be a reason for the particularly successful spread of the serovar in poultry and the low prevalence in pig, cattle and humans. However, fimbrial clusters which showed effects on intestinal colonization and persistence in mice were present in serovar 4,12:d:- (20).

The impact of feed contaminated with serovar 4,12:d:- on the infection of poultry seems to be low but can not be excluded. The percentage of serovar 4,12:d:- strains isolated from feed received at the NRL-Salmonella was only 1.4% during the last year. However, identical PFGE and virulence array type (VAT) profiles were found in isolates from feed and chickens, indicating the possible spread of this serovar by feeding stuffs, but it remains unclear if they are contaminated at the feed mill or secondarily at the poultry premises.

Microarray data revealed that serovar 4,12:d:- strains lacked several metabolism genes. Metabolism genes mostly related to anabolic or catabolic processes in bacterial cells are of major significance due to their ubiquitous spread as well as their essential functions. Some of these enzymes are detected by the array highlighting important pathways like glycolysis,

glyconeogenesis and pentose phosphate way or detecting genes encoding enzymes processing important intermediate products like *dgoA*, *pflD* or STM3782. Notably, *dgoA* was absent in all serovar 4,12:d:- strains and *pflD* was absent in all serovar 4,12:d:- strains isolated from chicken, pig and feed but was present in serovar 4,12:d:- strains isolated from humans. The *dgoA* gene encodes 2-oxo-3-deoxygalactonate-6-phosphate aldolase-galactonate dehydratase forming D-glyceraldehyde-3P one of the central metabolites produced in the galactonate metabolism. The role of *dgoA* is currently not well understood. The lack of this enzyme could have a toxic effect as shown for *E. coli* K12 (140) because of 2-keto-3-deoxy-D-galactonate 6-phosphate accumulation. Moreover, a lack of this enzyme could have a negative effect on the energy production of strains, leading to a slower cell growth because it hampers the production of D-glyceraldehyde-3P, an important intermediate product of the glycolysis and glyconeogenesis (203). A slower growth of these strains could also be caused by the absence of the pyruvate formate lyase encoded by *pflD* in serovar 4,12:d:-. This enzyme catalyzes the formation of acetyl-coenzyme A in the presence of pyruvate (211). Another metabolic gene STM3782 was absent in 6 out of 21 strains. This gene is involved in the phosphotransferase-system (PTS). PTS, described first 1964 (128), occurs in many gram-positive and gram-negative bacteria but not within archaea or eukaryotes (218). The PTS signal transduction joins aligned pathways of glycolysis, glyconeogenesis and the pentose phosphate way because the phosphorylation status of the central protein kinase is dependent on the purvate / phosphoenol pyruvate (PEP) concentration which itself depends on the amount of metabolic substrates metabolised within glycolysis and glyconeogenesis. A mutation analysis showed a significant lower intracellular growth rate of serovar Typhimurium compromised by a PTS mutation in mice (124).

Any genes which were preferentially present in serovar 4,12:d:- isolates from animals could not be identified.

In conclusion, serovar 4,12:d:- lacked several genes with known contributions to pathogenicity, metabolism and antimicrobial resistance in comparison to serovars which are highly prevalent in human and animals, e.g. serovars Enteritidis or Typhimurium. The absence of such genes might cause the low infection rate in humans, despite the prevalence in German chickens being high. Apparently, serovar 4,12:d:- possesses genetic factors which facilitate the colonization of chickens. This hypothesis is supported by the observation that the most prevalent PFGE profile 1 in chickens was only rarely found in human isolates. However, identical serovar 4,12:d:- virulence array types were observed in isolates from both hosts. The analysis of serovar 4,12:d:- strains isolated 10 years ago and of contemporary isolates showed

a low genetic diversity being a sign of the temporal persistence of a highly clonal line in German broilers. Altogether, epidemiological and molecular data show that serovar 4,12:d:- can pass through the food chain from feed to poultry and finally to humans occasionally causing salmonellosis. The virulence and resistance gene repertoire of serovar 4,12:d:- currently does not give reasons to expect that the serovar will pose a similar risk to consumers like other poultry associated serovars, especially *S. enterica* subsp. *enterica* serovars Enteritidis, Infantis or Hadar. However, serovar 4,12:d:- should be under supervision by routine public health and veterinary institutes. This will ensure the detection of the spread of the zoonosis into other countries and possibly identify changes to a higher prevalence in humans.

4.2.2 Application of the Salmonella *DNA microarray to study the gene repertoire in* S. enterica *serovar Paratyphi B dT+*

S. enterica serovar Paratyphi B dT+ related outbreaks have occurred all over the world (8,54,82,228). The serovar has been recognised as one of the most frequently isolated serovars in European broiler flocks especially in the Netherlands, in Belgium and Germany (65,87,109,242). In-vivo challenge experiments showed that this particular variant is efficiently transmitted within broilers and persisted until slaughter indicating a good adaptation to poultry (164,165). In this study the multi-drug resistance profile, the clonality and the pathogenic as well as resistance molecular gene repertoire of this particular broiler industry-associated serovar Paratyphi B dT+ clone were characterised and compared to serovar Paratyphi B dT+ isolates from other origins and sources.

Molecular characterisation of German isolates of this distinct multi-drug resistant serovar Paratyphi B dT+ clone have been previously described (165). Typically, they harbour a chromosomally located class 2 integron with *dfrA1-sat1-aadA1* resistance gene cassettes conferring resistance to trimethoprim, spectinomycin and streptomycin. Occasionally, they possess in addition a class 1 integron located on a 128-MDa transferable plasmid (165) with the *dfrA*1-*aadA1* resistance gene cassettes. These characteristics could be confirmed in all of the contemporary isolates from the Netherlands, Belgium and Germany.

Consequently, this multi-drug resistant clone has continuously spread throughout broiler flocks of these three European countries. All strains also exhibit a unique X8 *Xba*I PFGE pattern. The same PFGE profile was also found in isolates from pigs, humans, spice and animal feed ingredients indicating that the clone is able to persist not only in broilers but also

in the environment and can spread to pigs and humans (Brown *et al.*, 2003). Additional antimicrobial resistance has been observed such as against ampicillin (encoded by $bla_{\text{TEM1-like}}$), nalidixic acid, kanamycin and neomycin (encoded by *aphA1*), ceftiofur (encoded by $bla_{\text{CTX-M2}}$ and bla_{TEM52}). Resistance against 3rd generation cephalosporins was found in two recently isolated German strains and one Belgian strain which also possessed resistance to nalidixic acid. Selective pressure associated with regular medication of broilers for therapeutic or prophylactic purposes may lead to an accumulation of new resistance determinants including antimicrobial classes of critical importance for human therapy. This is especially true when producers try to eradicate *Salmonella* from their flocks by using newer antimicrobials via drinking water. Consequently, the number of serovar Paratyphi B dT+ isolates showing resistance to clinically important antimicrobials (fluoroquinolones and extended spectrum *ß*-lactams) seems to be increasing. During 2008 five Paratyphi B O:5- dT+ isolates belonging to the distinct clone (PFGE pattern X8, see Fig. 14) were received at the National *Salmonella* Reference Laboratory from various locations in Germany showing resistance to 3rd generation cephalosporins. Formerly no such isolates had been recognised.

A striking property of the particular clone, which is associated with broiler production, was the phenotypic and genotypic absence of the O:5 antigen. In contrast, several serovar Paratyphi B dT+ isolates originating from England and the Netherlands and isolates of the monophasic serovar 4,5,12:b:- lacking the phase-2 flagellar antigen compared to serovar Paratyphi B dT+ exhibited the O:5 antigen. Apart from one strain these were not isolated from poultry but from human, cattle, animal feed ingredients, and a dog. Isolates from cattle were previously obtained from an isolated herd outbreak involving clinically diseased calves and sub-clinical infection in calves and adult dairy cattle. The herd infection cleared spontaneously within months despite a tendency of the organism to persist in surface water on the farm (68). The O:5+ cluster of isolates represent a significantly more heterogeneous group with respect to their resistance profiles and PFGE patterns compared to the O:5- isolates (Fig. 14).

Three out of the seven serovar Paratyphi B O:5+ dT+ and all five serovar 4,5,12:b:- strains showed susceptibility to all 17 antimicrobials tested. Of the four multi-drug resistant serovar Paratyphi B O:5+ dT+ isolates, three harboured the *Salmonella* Genomic Island 1 previously described for serovar Typhimurium DT104 (30). They encode resistance to ampicillin (and amoxicillin) (encoded by bla_{PSE1}), chloramphenicol and florfenicol (encoded by *floR*), streptomycin and spectinomycin (encoded by *aadA2*), sulfamethoxazole (encoded by *sul1*) and tetracycline (encoded by *tet*(G)), described as the typical ACSSuT penta-resistance

phenotype (257). Such serovar Paratyphi B dT+ strains possessing a SGI-1 island have already been reported from fish tanks in Australia (133), humans in France (257), and calves in England (68). The SGI-1 positive isolates in this study were obtained from a human case in the Netherlands and two from bovine cases in England. The various places of isolation worldwide indicate the widespread nature of this particular serovar Paratyphi B O:5+ dT+ clone. All three isolates possessed an identical X17 PFGE profile (Fig. 14). One serovar Paratyphi B O:5+ dT+ strain (07-01981) isolated from chicken harbours an identical resistance profile (*bla*$_{\text{TEM1-like}}$-*dfrA1-sat1-aadA1*) like the typical serovar Paratyphi B O:5- dT+ clone. Apparently, this particular O:5+ clone might have adopted multi-drug resistance by horizontal gene transfer from the serovar Paratyphi B O:5- dT+ clone.

The heterogeneous serovar Paratyphi B O:5+ dT+ group including the serovar 4,5,12:b:- expressed the O:5 antigen encoded by the *oafA* gene. The lipopolysaccharide (LPS) O-antigen acetylase of serovar Typhimurium, designated *oafA,* is required for the O-acetylation of the 2-hydroxyl residue of the abequose moiety of inner-core LPS. The acetylation state of LPS has major effects on the immune response of the host, but no effects on virulence itself (222). Moreover, it was shown that the O-antigen acetylase changes the O-antigen structure significantly by alternating several conformational epitopes having great influence on host-microbe interaction.

The serovar Paratyphi B O:5+ dT+ group encodes a number of additional virulence genes, e.g. the fimbrial genes, prophage encoded genes and other virulence genes, compared to the O:5- group (Table 13). These additional genes might be a reason for the successful worldwide spread of the serovar Paratyphi B O:5+ dT+ group of probable reptilian or amphibian origin to multiple host species via international trade in contaminated feed ingredients and foodstuffs. For example, a comprehensive repertoire of fimbrial clusters enhances the chance to mediate adhesion to many hosts. With respect to serovar Paratyphi B O:5- dT+, serovar Paratyphi B O:5+ dT+ possesses three additional fimbrial clusters, namely *lpfD*, *stcC* and *stjB*. All three clusters occur also in serovar Typhimurium, which is known to have a broad host range and worldwide distribution. Fimbriae are responsible for the initial adhesion of the bacterium to eukaryotic cells and for some it was shown that they are required for full virulence (20,256). Similarly, several serovar Paratyphi B O:5+ dT+ isolates possess virulence plasmid encoded *spv* genes playing a role in systemic virulence in mice (136). Virulence plasmids might be an advantage in infection of certain hosts (250). The monophasic serovar 4,5,12:b:- strains harboured similar characteristics to serovar Paratyphi B O:5+ dT+ strains and could not be differentiated by the presence or absence of virulence genes (Fig. 15).

This indicates that both serovars are genetically closely related and evolved from a common ancestor. In contrast serovar Paratyphi B O:5- dT+ isolates showed identical PFGE profiles indicating a highly homologous clonal line. DNA microarray results supported this observation. All strains of the O:5- group from chicken, pig, human, the environment, and spice showed exactly the same gene content except the presence or absence of *sugR*. The gene *sugR* encodes for ATP binding protein on SPI-3. Potential deletions within SPI-3 have already been recognised in other serovars (27). It was shown that SPI-3 consists of a mosaic structure indicating a multi-step evolutionary process resulting in variations of SPI-3.

Prager *et al.* (192) defined various systemic (*d*-tartrate non-fermenting) and enteric (*d*-tartrate fermenting) *Salmonella enterica* subsp. *enterica* Paratyphi B pathovars based on the presence or absence of the virulence genes *sopE1*, *avrA*, *sopB*, *sopD* and *sptP*. For serovar Paratyphi B dT+ four enteric pathovars variants (EPVs) were defined. According to that classification all isolates from the particular O:5- group were grouped into the EPV3 with absence of the genes *sopE1* and *sopD* and presence of the genes *avrA*, *sopB*, and *sptP*. Serovar Paratyphi B O:5+ dT+ isolates could be grouped as EPV2 lacking the *avrA* and *sopE1* gene or as EPV1, exclusively lacking *sopE1*. All three SGI-1 positive Paratyphi B O:5+ dT+ isolates belonged to EPV2. Systemic pathovars defined by the lack of *avrA* but presence of *sopE1* were not observed in serovars Paratyphi B dT+ strains or 4,5,12:b:- strains supporting the proposal of the authors to use *sopE1* and *avrA* as an indicator for detecting systemic variants since all human isolates originated from cases of gastroenteritis.

In conclusion serovar Paratyphi B dT+ isolates are grouped into two main clusters that are phenotypically distinguishable by the expression of the O:5 antigen. On the molecular level serovar Paratyphi B O:5+ dT+ isolates reveal a variable genetic background compared to highly similar genetic background of the Paratyphi B O:5- dT+ isolates. The distribution of the virulence and resistance genes indicates that each group evolved from a different lineage.

4.3 Outlook

Within the last decade, DNA microarray technology has been rapidly adopted by many fields and emerged as a prominent research tool. In this thesis, DNA microarray technology was utilized in two separate studies together with supporting molecular typing methods to understand more about the genomic content of two *Salmonella enterica* serovars and their potential risk for humans. This combined approach of classical and new high performance applications forms a powerful tool to improve understanding an insight into the potential risk

for consumers along the food chain and to perform epidemiological analysis (including outbreak studies) for *Salmonella* in the future in an efficient, timely manner and at a reasonable cost. One of the future challenges will be to analyse data in a more convenient way and to further improve interpretation of the meaning of results.

The ongoing research into *Salmonella* necessitates further development of the DNA microarray. Old probes resulting in uncertain, false negative or (due to cross hybridisation) false positive results need to be redesigned and as well, newly discovered and published genes need to be implemented. Moreover, an analysis of the other marker groups should be performed in order to complete the overall analysis and probably reveal new combinations of genes in particular hosts. The analysis of the IS-elements may in particular be a new approach for typing *Salmonella* serovars. This has to be elucidated in the future. Another future approach would be the application of the DNA microarray in other food chains, e.g. pig, seafood, cattle or particular vegetables often linked to *Salmonella* outbreaks. Based upon the results obtained so far, further in-vitro and in-vivo studies which could illustrate in greater detail the implication of specific serovars for human salmonellosis should be performed. Infection studies disclosing the connection between virulence gene repertoire and infectiousness would be an approach with considerable benefits for levels of food safety.

The technological platforms of microarrays are still a fast developing field. Owing to the extensive validation of the 281 oligonucleotide probes in this study, the set could be easily adapted to other or forthcoming more cost effective platforms. To reduce costs, the current fluorescence DNA labelling could be replaced by biotin DNA labelling and subsequent detection with a streptavidin-gold conjugate complex as described by Sachse *et al*. (206). Single glass slides as carrier for the probes could be replaced by platforms with a higher-throughput. Currently, the DNA microarray prototype developed in this study is under inter-laboratory validation to show its robustness and reliability.

5. References

1. **Aarts, H. J. M., K. S. Boumedine, X. Nesme, and A. Cloeckaert**. 2001. Molecular tools for the characterisation of antibiotic-resistant bacteria. Vet. Res. **32**:363-380.

2. **Ahmer, B. M., R. J. van, P. R. Watson, T. S. Wallis, and F. Heffron**. 1999. *Salmonella* SirA is a global regulator of genes mediating enteropathogenesis. Mol. Microbiol. **31**:971-982.

3. **Akiba, M., K. Nakamura, D. Shinoda, N. Yoshii, H. Ito, I. Uchida, and M. Nakazawa**. 2006. Detection and characterization of variant *Salmonella* genomic island 1s from *Salmonella* Derby isolates. Jpn. J. Infect. Dis. **59**:341-345.

4. **Althouse, C., S. Patterson, P. Fedorka-Cray, and R. E. Isaacson**. 2003. Type 1 fimbriae of *Salmonella enterica* serovar Typhimurium bind to enterocytes and contribute to colonization of swine in vivo. Infect. Immun. **71**:6446-6452.

5. **Amavisit, P., D. Lightfoot, G. F. Browning, and P. F. Markham**. 2003. Variation between pathogenic serovars within *Salmonella* pathogenicity islands. J. Bacteriol. **185**:3624-3635.

6. **Anderson, E. S. and R. E. Williams**. 1956. Bacteriophage typing of enteric pathogens and staphylococci and its use in epidemiology. J. Clin. Pathol. **9**:94-127.

7. **Anonymous**. 2007. The EFSA Zoonoses Data Collection Reports http://www.efsa.europa.eu/EFSA/ScientificPanels/ZOONOSES/efsa_locale-1178620753812_ZoonosesDataCollection.htm.

8. **Anonymous**. 2008. Multistate Outbreak of human *Salmonella* infections associated with exposure to turtles --- United states, 2007-2008. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5703a3.htm.

9. **Anonymous**. 2009. Antibiotic Resistance in bacteria of animal origin. http://cordis.europa.eu/data/PROJ_FP5/ACTIONeqDndSESSIONeq112482005919ndDOCeq169ndTBLeqEN_PROJ.htm.

10. **Antunes, P., J. Machado, J. C. Sousa, and L. Peixe**. 2005. Dissemination of sulfonamide resistance genes (*sul1*, *sul2*, and *sul3*) in Portuguese *Salmonella enterica* strains and relation with integrons. Antimicrob. Agents Chemother. **49**:836-839.

11. **Arrach, N., S. Porwollik, P. Cheng, A. Cho, F. Long, S. H. Choi, and M. McClelland**. 2008. *Salmonella* serovar identification using PCR-based detection of gene presence and absence. J. Clin. Microbiol. **46**:2581-2589.

12. **Bajaj, V., R. L. Lucas, C. Hwang, and C. A. Lee**. 1996. Co-ordinate regulation of *Salmonella typhimurium* invasion genes by environmental and regulatory factors is mediated by control of hilA expression. Mol. Microbiol. **22**:703-714.

13. **Bakshi, C. S., V. P. Singh, M. W. Wood, P. W. Jones, T. S. Wallis, and E. E. Galyov**. 2000. Identification of SopE2, a *Salmonella* secreted protein which is highly homologous to SopE and involved in bacterial invasion of epithelial cells. J. Bacteriol. **182**:2341-2344.

14. **Barak, J. D., L. Gorski, P. Naraghi-Arani, and A. O. Charkowski**. 2005. *Salmonella enterica* virulence genes are required for bacterial attachment to plant tissue. Appl. Environ. Microbiol. **71**:5685-5691.

15. **Batchelor, M., K. L. Hopkins, E. Liebana, P. Slickers, R. Ehricht, M. Mafura, F. Aarestrup, D. Mevius, F. A. Clifton-Hadley, M. J. Woodward, R. H. Davies, E. J. Threlfall, and M. F. Anjum**. 2008. Development of a miniaturised microarray-based assay for the rapid identification of antimicrobial resistance genes in Gram-negative bacteria. Int. J. Antimicrob. Agents .

16. **Baucheron, S., H. Imberechts, E. Chaslus-Dancla, and A. Cloeckaert**. 2002. The AcrB multidrug transporter plays a major role in high-level fluoroquinolone resistance in *Salmonella enterica* serovar Typhimurium phage type DT204. Microb. Drug Resist. **8**:281-289.

17. **Bäumler, A. J. and F. Heffron**. 1995. Identification and sequence analysis of *lpfABCDE*, a putative fimbrial operon of *Salmonella typhimurium*. J. Bacteriol. **177**:2087-2097.

18. **Bäumler, A. J., J. G. Kusters, I. Stojiljkovic, and F. Heffron**. 1994. *Salmonella typhimurium* loci involved in survival within macrophages. Infect. Immun. **62**:1623-1630.

19. **Bäumler, A. J., R. M. Tsolis, F. A. Bowe, J. G. Kusters, S. Hoffmann, and F. Heffron**. 1996. The *pef* fimbrial operon of *Salmonella typhimurium* mediates adhesion to murine small intestine and is necessary for fluid accumulation in the infant mouse. Infect. Immun. **64**:61-68.

20. **Bäumler, A. J., R. M. Tsolis, and F. Heffron**. 1996. The *lpf* fimbrial operon mediates adhesion of *Salmonella typhimurium* to murine Peyer's patches. Proc. Natl. Acad. Sci. U.S.A **93**:279-283.

21. **Behr, M. A., M. A. Wilson, W. P. Gill, H. Salamon, G. K. Schoolnik, S. Rane, and P. M. Small**. 1999. Comparative genomics of BCG vaccines by whole-genome DNA microarray. Science **284**:1520-1523.

22. **Benson, D. A., I. Karsch-Mizrachi, D. J. Lipman, J. Ostell, and D. L. Wheeler**. 2008. GenBank. Nucleic Acids Res. **36**:D25-D30.

23. **Berghold, C., C. Kornschober, and S. Weber**. 2003. A regional outbreak of S. Enteritidis phage type 5, traced back to the flocks of an egg producer, Austria. Euro Surveill. **8**:195-198.

24. **Bertrand, S., R. Rimhanen-Finne, F. X. Weill, W. Rabsch, L. Thornton, J. Perevoscikovs, W. van Pelt, and M. Heck**. 2008. *Salmonella* infections associated with reptils: the current situation in Europe. Euro. Surveill. **13**:pii=18902.

25. **Bijlsma, J. J. and E. A. Groisman**. 2005. The PhoP/PhoQ system controls the intramacrophage type three secretion system of *Salmonella enterica*. Mol. Microbiol. **57**:85-96.

26. **Blanc-Potard, A. B. and E. A. Groisman**. 1997. The *Salmonella selC* locus contains a pathogenicity island mediating intramacrophage survival. EMBO J. **16**:5376-5385.

27. **Blanc-Potard, A. B., F. Solomon, J. Kayser, and E. A. Groisman**. 1999. The SPI-3 pathogenicity island of *Salmonella enterica*. J. Bacteriol. **181**:998-1004.

28. **Blaser, M. J. and L. S. Newman**. 1982. A review of human salmonellosis: I. Infective dose. Rev. Infect. Dis. **4**:1096-1106.

29. **Bodrossy, L. and A. Sessitsch**. 2004. Oligonucleotide microarrays in microbial diagnostics. Curr. Opin. Microbiol. **7**:245-254.

30. **Boyd, D., A. Cloeckaert, E. Chaslus-Dancla, and M. R. Mulvey**. 2002. Characterization of variant *Salmonella* genomic island 1 multidrug resistance regions from serovars Typhimurium DT104 and Agona. Antimicrob. Agents Chemother. **46**:1714-1722.

31. **Boyd, D., G. A. Peters, A. Cloeckaert, K. S. Boumedine, E. Chaslus-Dancla, H. Imberechts, and M. R. Mulvey**. 2001. Complete nucleotide sequence of a 43-kilobase genomic island associated with the multidrug resistance region of *Salmonella enterica* serovar Typhimurium DT104 and its identification in phage type DT120 and serovar Agona. J. Bacteriol. **183**:5725-5732.

32. **Brown, D. J., L. M. Browning, and J. E. Coica**. 2003. Investigation of human infections with *Salmonella enterica* serovar Java in Scotland and possible association with imported poultry. Eurosurveilance **8**:35-40.

33. **Bunny, K., J. Liu, and J. Roth**. 2002. Phenotypes of *lexA* mutations in *Salmonella enterica*: evidence for a lethal *lexA* null phenotype due to the Fels-2 prophage. J. Bacteriol. **184**:6235-6249.

34. **Butaye, P., A. Cloeckaert, and S. Schwarz**. 2003. Mobile genes coding for efflux-mediated antimicrobial resistance in Gram-positive and Gram-negative bacteria. Int. J. Antimicrob. Agents **22**:205-210.

35. **Callow, B. R.** 1959. A new phage-typing scheme for *Salmonella typhimurium*. J. Hyg. (Lond) **57**:346-359.

36. **Carattoli, A., A. Bertini, L. Villa, V. Falbo, K. L. Hopkins, and E. J. Threlfall**. 2005. Identification of plasmids by PCR-based replicon typing. J. Microbiol. Methods **63**:219-228.

37. **Carattoli, A., E. Filetici, L. Villa, A. M. Dionisi, A. Ricci, and I. Luzzi**. 2002. Antibiotic resistance genes and *Salmonella* genomic island 1 in *Salmonella enterica* serovar Typhimurium isolated in Italy. Antimicrob. Agents Chemother. **46**:2821-2828.

38. **Chakravortty, D., I. Hansen-Wester, and M. Hensel**. 2002. *Salmonella* pathogenicity island 2 mediates protection of intracellular *Salmonella* from reactive nitrogen intermediates. J. Exp. Med. **195**:1155-1166.

39. **Cheung, V. G., M. Morley, F. Aguilar, A. Massimi, R. Kucherlapati, and G. Childs**. 1999. Making and reading microarrays. Nat. Genet. **21**:15-19.

40. **Chiu, C. H., H. L. Chen, L. S. Kao, C. Y. Yang, C. Chu, B. Doublet, K. Praud, and A. Cloeckaert**. 2007. Variant *Salmonella* genomic island 1 antibiotic resistance

gene clusters in *Salmonella enterica* serovar Derby isolates from humans in Taiwan. J. Antimicrob. Chemother. **59**:325-326.

41. **Chu, C., C. H. Chiu, W. Y. Wu, C. H. Chu, T. P. Liu, and J. T. Ou**. 2001. Large drug resistance virulence plasmids of clinical isolates of *Salmonella enterica* serovar Choleraesuis. Antimicrob. Agents Chemother. **45**:2299-2303.

42. **Chu, C., S. F. Hong, C. Tsai, W. S. Lin, T. P. Liu, and J. T. Ou**. 1999. Comparative physical and genetic maps of the virulence plasmids of *Salmonella enterica* serovars Typhimurium, Enteritidis, Choleraesuis, and Dublin. Infect. Immun. **67**:2611-2614.

43. **Chuang, Y. C., K. C. Wang, Y. T. Chen, C. H. Yang, S. C. Men, C. C. Fan, L. H. Chang, and K. S. Yeh**. 2008. Identification of the genetic determinants of *Salmonella enterica* serotype Typhimurium that may regulate the expression of the type 1 fimbriae in response to solid agar and static broth culture conditions. BMC. Microbiol. **8**:126.

44. **Cirillo, D. M., R. H. Valdivia, D. M. Monack, and S. Falkow**. 1998. Macrophage-dependent induction of the *Salmonella* pathogenicity island 2 type III secretion system and its role in intracellular survival. Mol. Microbiol. **30**:175-188.

45. **Clark, N. C., O. Olsvik, J. M. Swenson, C. A. Spiegel, and F. C. Tenover**. 1999. Detection of a streptomycin/spectinomycin adenylyltransferase gene (*aadA*) in *Enterococcus faecalis*. Antimicrob. Agents Chemother. **43**:157-160.

46. **Clegg, S. and G. F. Gerlach**. 1987. Enterobacterial fimbriae. J. Bacteriol. **169**:934-938.

47. **Cloeckaert, A. and S. Schwarz**. 2001. Molecular characterization, spread and evolution of multidrug resistance in *Salmonella enterica* Typhimurium DT104. Vet. Res. **32**:301-310.

48. **Collazo, C. M. and J. E. Galán**. 1997. The invasion-associated type-III protein secretion system in *Salmonella* - A review. Gene **192**:51-59.

49. **Cooke, F. J., D. J. Brown, M. Fookes, D. Pickard, A. Ivens, J. Wain, M. Roberts, R. A. Kingsley, N. R. Thomson, and G. Dougan**. 2008. Characterization of the genomes of a diverse collection of *Salmonella enterica* serovar Typhimurium definitive phage type 104. J. Bacteriol. **190**:8155-8162.

50. **Crespo, P. S., G. Hernandez, A. Echeita, A. Torres, P. Ordonez, and A. Aladuena**. 2005. Surveillance of foodborne disease outbreaks associated with consumption of eggs and egg products: Spain, 2002-2003. Euro Surveill. **10**:E050616.2.

51. **del Cerro, A., S. M. Soto, E. Landeras, M. A. Gonzalez-Hevia, J. A. Guijarro, and M. C. Mendoza**. 2002. PCR-based procedures in detection and DNA-fingerprinting of *Salmonella* from samples of animal origin. Food Microbiol. **19**:567-575.

52. **del, S. G., R. Giraldo, M. J. Ruiz-Echevarria, M. Espinosa, and R. az-Orejas**. 1998. Replication and control of circular bacterial plasmids. Microbiol. Mol. Biol. Rev. **62**:434-464.

53. **Denny, J., J. Threlfall, J. Takkinen, S. Löfdahl, T. Westrell, C. Varela, B. Adak, N. Boxall, S. Ethelberg, M. Torpdahl, M. Straetemans, and W. van Pelt**. 2007. Multinational *Salmonella* Paratyphi B variant Java (*Salmonella* Java) outbreak, August - December 2007. Euro Surveill. **12**:2-6.

54. **Desenclos, J. C., P. Bouvet, E. Benz-Lemoine, F. Grimont, H. Desqueyroux, I. Rebiere, and P. A. Grimont**. 1996. Large outbreak of *Salmonella enterica* serotype Paratyphi B infection caused by a goats' milk cheese, France, 1993: a case finding and epidemiological study. BMJ **312**:91-94.

55. **Doffinger, R., S. Patel, and D. S. Kumararatne**. 2005. Human immunodeficiencies that predispose to intracellular bacterial infections. Curr. Opin. Rheumatol. **17**:440-446.

56. **Doublet, B., D. Boyd, M. R. Mulvey, and A. Cloeckaert**. 2005. The *Salmonella* genomic island 1 is an integrative mobilizable element. Mol. Microbiol. **55**:1911-1924.

57. **Doublet, B., R. Lailler, D. Meunier, A. Brisabois, D. Boyd, M. R. Mulvey, E. Chaslus-Dancla, and A. Cloeckaert**. 2003. Variant *Salmonella* genomic island 1 antibiotic resistance gene cluster in *Salmonella enterica* serovar Albany. Emerg. Infect. Dis. **9**:585-591.

58. **Duguid, J. P., E. S. Anderson, and I. Campbell**. 1966. Fimbriae and adhesive properties in Salmonellae. J. Pathol. Bacteriol. **92**:107-138.

59. **Duguid, J. P., M. R. Darekar, and D. W. Wheater**. 1976. Fimbriae and infectivity in *Salmonella typhimurium*. J. Med. Microbiol. **9**:459-473.

60. **Edsall, G., S. Gaines, M. Landy, W. D. Tigertt, H. Sprinz, R. J. Trapani, A. D. Mandel, and A. S. Benenson**. 1960. Studies on infection and immunity in experimental typhoid fever. I. Typhoid fever in chimpanzees orally infected with *Salmonella typhosa*. J. Exp. Med. **112**:143-166.

61. **Edwards, R. A., G. J. Olsen, and S. R. Maloy**. 2002. Comparative genomics of closely related salmonellae. Trends Microbiol. **10**:94-99.

62. **EFSA**. 2009. The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2007, The EFSA Journal **223**.

63. **Eriksson, S., S. Lucchini, A. Thompson, M. Rhen, and J. C. Hinton**. 2003. Unravelling the biology of macrophage infection by gene expression profiling of intracellular *Salmonella enterica*. Mol. Microbiol. **47**:103-118.

64. **European Food Saftey Authority (EFSA)**. 2007. Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline study on the prevalence of *Salmonella* in holdings of laying hen flocks of *Gallus gallus*. The EFSA Journal **97**.

65. **European Food Saftey Authority (EFSA)**. 2007. Report of the Task Force on Zoonoses data collection on the analysis of the baseline survey on the prevalence of *Salmonella* in broiler flocks of *Gallus gallus*, in the EU, 2005-2006. The EFSA Journal **98**:1--85.

66. **European Food Saftey Authority (EFSA)**. 2007. Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline survey on the prevalence of *Salmonella* in slaughter pigs, in the EU, 2006-2007[1] - Part A: *Salmonella* prevalence estimates. The EFSA Journal **97**.

67. **European Food Saftey Authority (EFSA)**. 2008. Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline survey on the prevalence of *Salmonella* in turkey flocks, in the EU, 2006-2007 - Part A: *Salmonella* prevalence estimates. The EFSA Journal **134**:1-194.

68. **Evans, S. J., R. H. Davies, S. H. Binns, E. Liebana, T. W. Jones, M. F. Millar, E. J. Threlfall, L. R. Ward, K. L. Hopkins, P. H. Mackay, and P. J. Gayford**. 2005. Multiple antimicrobial resistant *Salmonella enterica* serovar Paratyphi B variant Java in cattle: a case report. Vet. Rec. **156**:343-346.

69. **Fierer, J.** 2001. Extra-intestinal *Salmonella* infections: the significance of *spv* genes. Clin.Infect.Dis. **32**:519-520.

70. **Figueroa-Bossi, N. and L. Bossi**. 1999. Inducible prophages contribute to *Salmonella* virulence in mice. Mol. Microbiol. **33**:167-176.

71. **Figueroa-Bossi, N., S. Uzzau, D. Maloriol, and L. Bossi**. 2001. Variable assortment of prophages provides a transferable repertoire of pathogenic determinants in *Salmonella*. Mol. Microbiol. **39**:260-271.

72. **Fisher, I. S.** 2004. Dramatic shift in the epidemiology of *Salmonella enterica* serotype Enteritidis phage types in Western Europe, 1998-2003--results from the Enter-net international *Salmonella* database. Euro Surveill. **9**:43-45.

73. **Fitzgerald, C., R. Sherwood, L. L. Gheesling, F. W. Brenner, and P. I. Fields**. 2003. Molecular analysis of the *rfb* O antigen gene cluster of *Salmonella enterica* serogroup O:6,14 and development of a serogroup-specific PCR assay. Appl. Environ. Microbiol. **69**:6099-6105.

74. **Foley, S. L., S. Zhao, and R. D. Walker**. 2007. Comparison of molecular typing methods for the differentiation of *Salmonella* foodborne pathogens. Foodborne Pathog. Dis. **4**:253-276.

75. **Fosse, J., H. Seegers, and C. Magras**. 2009. Prevalence and Risk Factors for Bacterial Food-Borne Zoonotic Hazards in Slaughter Pigs: A Review. Zoonoses Public Health [epub ahead of print].

76. **Frana, T. S., S. A. Carlson, and R. W. Griffith**. 2001. Relative distribution and conservation of genes encoding aminoglycoside- modifying enzymes in *Salmonella enterica* serotype typhimurium phage type DT104. Appl. Environ. Microbiol. **67**:445-448.

77. **Frech, G., C. Kehrenberg, and S. Schwarz**. 2003. Resistance phenotypes and genotypes of multiresistant *Salmonella enterica* subsp. *enterica* serovar Typhimurium var. Copenhagen isolates from animal sources. J. Antimicrob. Chemother. **51**:180-182.

78. **Frech, G. and S. Schwarz**. 2000. Molecular analysis of tetracycline resistance in *Salmonella enterica* subsp. *enterica* serovars Typhimurium, Enteritidis, Dublin,

Choleraesuis, Hadar and Saintpaul: construction and application of specific gene probes. J. Appl. Microbiol. **89**:633-641.

79. **Frenzen, P. D., Riggs, T. L., Buzby, J. C., Breuer, T., Roberts, T., Voetsch, D., and Reddy, S**. 1999. *Salmonella* cost estimate updates using FoodNet Data. Food Rev. **22**[2], 10-15.

80. **Friebel, A., H. Ilchmann, M. Aepfelbacher, K. Ehrbar, W. Machleidt, and W. D. Hardt**. 2001. SopE and SopE2 from *Salmonella typhimurium* activate different sets of RhoGTPases of the host cell. J. Biol. Chem. **276**:34035-34040.

81. **Garmendia, J., C. R. Beuzon, J. Ruiz-Albert, and D. W. Holden**. 2003. The roles of SsrA-SsrB and OmpR-EnvZ in the regulation of genes encoding the *Salmonella typhimurium* SPI-2 type III secretion system. Microbiology **149**:2385-2396.

82. **Gaulin, C., C. Vincent, L. Alain, and J. Ismail**. 2002. Outbreak of *Salmonella paratyphi* B linked to aquariums in the province of Quebec, 2000. Can. Commun. Dis. Rep. **28**:89-93, 96.

83. **Goldstein, C., M. D. Lee, S. Sanchez, C. Hudson, B. Phillips, B. Register, M. Grady, C. Liebert, A. O. Summers, D. G. White, and J. J. Maurer**. 2001. Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. Antimicrob. Agents Chemother. **45**:723-726.

84. **Gray, J. T., T. J. Stabel, and P. J. Fedorka-Cray**. 1996. Effect of dose on the immune response and persistence of *Salmonella choleraesuis* infection in swine. Am. J. Vet. Res. **57**:313-319.

85. **Grimont, P. A. D. and F.-X. Weill**. 2007. Antigenic formulae of the *Salmonella* serovars. WHO Collaborating Centre for Reference and Research on *Salmonella* , Institut Pasteur, Paris, France.

86. **Grindley, N. D.** 1983. Transposition of Tn3 and related transposons. Cell **32**:3-5.

87. **Guard-Petter, J.** 2001. The chicken, the egg and *Salmonella enteritidis*. Environ. Microbiol. **3**:421-430.

88. **Guerra, B., E. Junker, A. Miko, R. Helmuth, and M. C. Mendoza**. 2004. Characterization and localization of drug resistance determinants in multidrug-resistant, integron-carrying *Salmonella enterica* serotype Typhimurium strains. Microb. Drug Resist. **10**:83-91.

89. **Guerra, B., I. Laconcha, S. M. Soto, M. A. Gonzalez-Hevia, and M. C. Mendoza**. 2000. Molecular characterisation of emergent multiresistant *Salmonella enterica* serotype [4,5,12:i:-] organisms causing human salmonellosis. FEMS Microbiol. Lett. **190**:341-347.

90. **Guerra, B., S. Soto, S. Cal, and M. C. Mendoza**. 2000. Antimicrobial resistance and spread of class 1 integrons among *Salmonella* serotypes. Antimicrob. Agents Chemother. **44**:2166-2169.

91. **Guerra, B., S. M. Soto, J. M. Arguelles, and M. C. Mendoza**. 2001. Multidrug resistance is mediated by large plasmids carrying a class 1 integron in the emergent *Salmonella enterica* serotype [4,5,12:i:-]. Antimicrob. Agents Chemother. **45**:1305-1308.

92. **Gulig, P. A.** 1990. Virulence plasmids of *Salmonella typhimurium* and other salmonellae. Microb. Pathog. **8**:3-11.

93. **Gunn, J. S., W. J. Belden, and S. I. Miller**. 1998. Identification of PhoP-PhoQ activated genes within a duplicated region of the *Salmonella typhimurium* chromosome. Microb. Pathog. **25**:77-90.

94. **Hacker, J., G. Blum-Oehler, I. Muhldorfer, and H. Tschape**. 1997. Pathogenicity islands of virulent bacteria: structure, function and impact on microbial evolution. Mol. Microbiol. **23**:1089-1097.

95. **Haneda, T., N. Okada, N. Nakazawa, T. Kawakami, and H. Danbara**. 2001. Complete DNA sequence and comparative analysis of the 50-kilobase virulence plasmid of *Salmonella enterica* serovar Choleraesuis. Infect. Immun. **69**:2612-2620.

96. **Hansen-Wester, I., D. Chakravortty, and M. Hensel**. 2004. Functional transfer of *Salmonella* pathogenicity island 2 to *Salmonella bongori* and *Escherichia coli*. Infect. Immun. **72**:2879-2888.

97. **Hansen-Wester, I. and M. Hensel**. 2002. Genome-based identification of chromosomal regions specific for *Salmonella* spp. Infect. Immun. **70**:2351-2360.

98. **Harbottle, H., D. G. White, P. F. McDermott, R. D. Walker, and S. Zhao**. 2006. Comparison of multilocus sequence typing, pulsed-field gel electrophoresis, and antimicrobial susceptibility typing for characterization of *Salmonella enterica* serotype Newport isolates. J. Clin. Microbiol. **44**:2449-2457.

99. **Hartung, M.** 2005. Epidemiologische Situation der Zoonosen in Deutschland im Jahr 2005. Bundesinstitut für Risikobewertung Hausdruckerei, Berlin.

100. **Heffernan, E. J., S. Reed, J. Hackett, J. Fierer, C. Roudier, and D. Guiney**. 1992. Mechanism of resistance to complement-mediated killing of bacteria encoded by the *Salmonella typhimurium* virulence plasmid gene *rck*. J. Clin. Invest **90**:953-964.

101. **Heller, M. J.** 2002. DNA microarray technology: devices, systems, and applications. Annu. Rev. Biomed. Eng **4**:129-153.

102. **Hensel, M.** 2004. Evolution of pathogenicity islands of *Salmonella enterica*. Int. J. Med. Microbiol. **294**:95-102.

103. **Hensel, M., A. P. Hinsley, T. Nikolaus, G. Sawers, and B. C. Berks**. 1999. The genetic basis of tetrathionate respiration in *Salmonella typhimurium*. Mol. Microbiol. **32**:275-287.

104. **Hensel, M., T. Nikolaus, and C. Egelseer**. 1999. Molecular and functional analysis indicates a mosaic structure of *Salmonella* pathogenicity island 2. Mol. Microbiol. **31**:489-498.

105. **Hensel, M., J. E. Shea, S. R. Waterman, R. Mundy, T. Nikolaus, G. Banks, A. Vazquez-Torres, C. Gleeson, F. C. Fang, and D. W. Holden**. 1998. Genes encoding putative effector proteins of the type III secretion system of *Salmonella* pathogenicity island 2 are required for bacterial virulence and proliferation in macrophages. Mol. Microbiol. **30**:163-174.

106. **Hernandez, L. D., K. Hueffer, M. R. Wenk, and J. E. Galan**. 2004. *Salmonella* modulates vesicular traffic by altering phosphoinositide metabolism. Science **304**:1805-1807.

107. **Ho, T. D., N. Figueroa-Bossi, M. Wang, S. Uzzau, L. Bossi, and J. M. Slauch**. 2002. Identification of GtgE, a novel virulence factor encoded on the Gifsy-2 bacteriophage of *Salmonella enterica* serovar Typhimurium. J. Bacteriol. **184**:5234-5239.

108. **Hopkins, K. L., M. J. Batchelor, M. Anjum, R. H. Davies, and E. J. Threlfall**. 2007. Comparison of antimicrobial resistance genes in nontyphoidal salmonellae of serotypes Enteritidis, Hadar, and Virchow from humans and food-producing animals in England and wales. Microb. Drug Resist. **13**:281-288.

109. **Huehn, S., C. Bunge, E. Junker, R. Helmuth, and B. Malorny**. 2008. Emerging poultry associated *Salmonella enterica* subsp. *enterica* serovar 4,12:d:- reveals high clonality and a distinct pathogenicity gene repertoire. Appl. Environ. Microbiol. **75**:1011-1020.

110. **Hughes, T. R., M. Mao, A. R. Jones, J. Burchard, M. J. Marton, K. W. Shannon, S. M. Lefkowitz, M. Ziman, J. M. Schelter, M. R. Meyer, S. Kobayashi, C. Davis, H. Dai, Y. D. He, S. B. Stephaniants, G. Cavet, W. L. Walker, A. West, E. Coffey, D. D. Shoemaker, R. Stoughton, A. P. Blanchard, S. H. Friend, and P. S. Linsley**. 2001. Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer. Nat. Biotechnol. **19**:342-347.

111. **Humphrey, T.** 2000. Public-health aspects of *Salmonella* infection, p. 245-263. *In* C. Way and A. Way (eds.), *Salmonella* in domestic animals. CABI Publishing, Oxon, UK.

112. **Hunter, S. B., P. Vauterin, M. A. Lambert-Fair, M. S. Van Duyne, K. Kubota, L. Graves, D. Wrigley, T. Barrett, and E. Ribot**. 2005. Establishment of a universal size standard strain for use with the PulseNet standardized pulsed-field gel electrophoresis protocols: converting the national databases to the new size standard. J. Clin. Microbiol. **43**:1045-1050.

113. **Huovinen, P., L. Sundström, G. Swedberg, and O. Sköld**. 1995. Trimethoprim and sulfonamide resistance. Antimicrob. Agents Chemother. **39**:279-289.

114. **Ide, N., T. Ikebe, and K. Kutsukake**. 1999. Reevaluation of the promoter structure of the class 3 flagellar operons of *Escherichia coli* and *Salmonella*. Genes Genet. Syst. **74**:113-116.

115. **Jones, B. D. and S. Falkow**. 1996. Salmonellosis: Host immune responses and bacterial virulence determinants. Annu.Rev.Immunol. **14**:533-561.

116. **Jones, G. W. and R. E. Isaacson**. 1983. Proteinaceous bacterial adhesins and their receptors. Crit. Rev. Microbiol. **10**:229-260.

117. **Joys, T. M.** 1985. The covalent structure of the phase-1 flagellar filament protein of *Salmonella typhimurium* and its comparison with other flagellins. J. Biol. Chem. **260**:15758-15761.

118. **Kane, M. D., T. A. Jatkoe, C. R. Stumpf, J. Lu, J. D. Thomas, and S. J. Madore**. 2000. Assessment of the sensitivity and specificity of oligonucleotide (50mer) microarrays. Nucleic Acids Res. **28**:4552-4557.

119. **Kang, H., C. Loui, R. I. Clavijo, L. W. Riley, and S. Lu**. 2006. Survival characteristics of *Salmonella enterica* serovar Enteritidis in chicken egg albumen. Epidemiol. Infect. **134**:967-976.

120. **Kapperud, G., S. Gustavsen, I. Hellesnes, A. H. Hansen, J. Lassen, J. Hirn, M. Jahkola, M. A. Montenegro, and R. Helmuth**. 1990. Outbreak of *Salmonella typhimurium* infection traced to contaminated chocolate and caused by a strain lacking the 60-megadalton virulence plasmid. J. Clin. Microbiol. **28**:2597-2601.

121. **Kidgell, C., U. Reichard, J. Wain, B. Linz, M. Torpdahl, G. Dougan, and M. Achtman**. 2002. *Salmonella typhi*, the causative agent of typhoid fever, is approximately 50,000 years old. Infect. Genet. Evol. **2**:39-45.

122. **Kingsley, R. A., A. D. Humphries, E. H. Weening, M. R. De Zoete, S. Winter, A. Papaconstantinopoulou, G. Dougan, and A. J. Bäumler**. 2003. Molecular and phenotypic analysis of the CS54 island of *Salmonella enterica* serotype Typhimurium: identification of intestinal colonization and persistence determinants. Infect. Immun. **71**:629-640.

123. **Knodler, L. A., B. A. Vallance, M. Hensel, D. Jackel, B. B. Finlay, and O. Steele-Mortimer**. 2003. *Salmonella* type III effectors PipB and PipB2 are targeted to detergent-resistant microdomains on internal host cell membranes. Mol. Microbiol. **49**:685-704.

124. **Kok, M., G. Bron, B. Erni, and S. Mukhija**. 2003. Effect of enzyme I of the bacterial phosphoenolpyruvate : sugar phosphotransferase system (PTS) on virulence in a murine model. Microbiology **149**:2645-2652.

125. **Korhonen, T. K., H. Leffler, and E. C. Svanborg**. 1981. Binding specificity of piliated strains of *Escherichia coli* and *Salmonella typhimurium* to epithelial cells, saccharomyces cerevisiae cells, and erythrocytes. Infect. Immun. **32**:796-804.

126. **Korsak, N., B. Jacob, B. Groven, G. Etienne, B. China, Y. Ghafir, and G. Daube**. 2003. *Salmonella* contamination of pigs and pork in an integrated pig production system. J. Food Prot. **66**:1126-1133.

127. **Kuhle, V., G. L. Abrahams, and M. Hensel**. 2006. Intracellular *Salmonella enterica* redirect exocytic transport processes in a *Salmonella* pathogenicity island 2-dependent manner. Traffic. **7**:716-730.

128. **Kundig, W., S. Ghosh, and S. Roseman**. 1964. Phosphate bound to histidine in a protein as an intermediate in a novel phospho-transferase system. Proc. Natl. Acad. Sci. U.S.A **52**:1067-1074.

129. **Lawley, T. D., K. Chan, L. J. Thompson, C. C. Kim, G. R. Govoni, and D. M. Monack**. 2006. Genome-wide screen for *Salmonella* genes required for long-term systemic infection of the mouse. PLoS. Pathog. **2**:e11.

130. **Le Minor, L.** 1984. Genus III. *Salmonella*, p. 427-458. *In* N. R. Krieg, D. H. Bergey, and J. G. Holt (eds.), Bergey's manual of systematic bacteriology. Baltimore, USA.

131. **Le Minor, L. and M. Y. Popoff**. 1987. Designation of *Salmonella enterica* sp. nov., nom. rev., as the type and only species of the genus *Salmonella*. Int. J. Syst. Bacteriol. **37**:465-468.

132. **Ledeboer, N. A., J. G. Frye, M. McClelland, and B. D. Jones**. 2006. *Salmonella enterica* serovar Typhimurium requires the Lpf, Pef, and Tafi fimbriae for biofilm formation on HEp-2 tissue culture cells and chicken intestinal epithelium. Infect. Immun. **74**:3156-3169.

133. **Levings, R. S., D. Lightfoot, R. M. Hall, and S. P. Djordjevic**. 2006. Aquariums as reservoirs for multidrug-resistant *Salmonella* Paratyphi B. Emerg. Infect. Dis. **12**:507-510.

134. **Levings, R. S., D. Lightfoot, S. R. Partridge, R. M. Hall, and S. P. Djordjevic**. 2005. The genomic island SGI1, containing the multiple antibiotic resistance region of *Salmonella enterica* serovar Typhimurium DT104 or variants of it, is widely distributed in other *S. enterica* serovars. J. Bacteriol. **187**:4401-4409.

135. **Liao, A. P., E. O. Petrof, S. Kuppireddi, Y. Zhao, Y. Xia, E. C. Claud, and J. Sun**. 2008. *Salmonella* type III effector AvrA stabilizes cell tight junctions to inhibit inflammation in intestinal epithelial cells. PLoS ONE **3**:e2369.

136. **Libby, S. J., L. G. Adams, T. A. Ficht, C. Allen, H. A. Whitford, N. A. Buchmeier, S. Bossie, and D. G. Guiney**. 1997. The *spv* genes on the *Salmonella dublin* virulence plasmid are required for severe enteritis and systemic infection in the natural host. Infect. Immun. **65**:1786-1792.

137. **Libby, S. J., M. Lesnick, P. Hasegawa, M. Kurth, C. Belcher, J. Fierer, and D. G. Guiney**. 2002. Characterization of the *spv* locus in *Salmonella* enterica serovar Arizona. Infect. Immun. **70**:3290-3294.

138. **Liebert, C. A., R. M. Hall, and A. O. Summers**. 1999. Transposon Tn*21*, flagship of the floating genome. Microbiol. Mol. Biol. Rev. **63**:507-522.

139. **Lim, S., B. Kim, H. S. Choi, Y. Lee, and S. Ryu**. 2006. Fis is required for proper regulation of *ssaG* expression in *Salmonella enterica* serovar Typhimurium. Microb. Pathog. **41**:33-42.

140. **Lin, E. C. C.** 1996. Dissimilatory pathways for sugars, polyols, and carboxylates, p. 307-342. *In* A. Böck, R. Curtiss III, J. B. Kaper, F. C. Neidhardt, T. Nyström, K. E. Rudd, and C. L. Squires (eds.), *Escherichia coli* and *Salmonella*. ASM Press, Washington, D.C.

141. **Lindstedt, B. A., E. Heir, I. Nygard, and G. Kapperud**. 2003. Characterization of class I integrons in clinical strains of *Salmonella enterica* subsp. *enterica* serovars Typhimurium and Enteritidis from Norwegian hospitals. J. Med. Microbiol. **52**:141-149.

142. **Liu, D., R. A. Cole, and P. R. Reeves**. 1996. An O-antigen processing function for Wzx (RfbX): a promising candidate for O-unit flippase. J. Bacteriol. **178**:2102-2107.

143. **Löfström, C., J. Eriksson, A. Aspan, P. Häggblom, A. Gunnarsson, E. Borch, and P. Radström**. 2006. Improvement and validation of RAPD in combination with PFGE analysis of *Salmonella enterica* ssp. *enterica* serovar Senftenberg strains isolated from feed mills. Vet. Microbiol. **114**:345-351.

144. **Lu, F. and N. L. Craig**. 2000. Isolation and characterization of Tn*7* transposase gain-of-function mutants: a model for transposase activation. EMBO J. **19**:3446-3457.

145. **Lucas, R. L. and C. A. Lee**. 2001. Roles of *hilC* and *hilD* in regulation of *hilA* expression in *Salmonella enterica* serovar Typhimurium. J. Bacteriol. **183**:2733-2745.

146. **Lukinmaa, S., R. Schildt, T. Rinttila, and A. Siitonen**. 1999. *Salmonella enteritidis* phage types 1 and 4: pheno- and genotypic epidemiology of recent outbreaks in Finland. J. Clin. Microbiol. **37**:2176-2182.

147. **Madsen, L., F. M. Aarestrup, and J. E. Olsen**. 2000. Characterisation of streptomycin resistance determinants in danish isolates of *Salmonella* Typhimurium. Vet. Microbiol. **75**:73-82.

148. **Mahillon, J. and M. Chandler**. 1998. Insertion sequences. Microbiol. Mol. Biol. Rev. **62**:725-774.

149. **Maiden, M. C.** 1998. Horizontal genetic exchange, evolution, and spread of antibiotic resistance in bacteria. Clin. Infect. Dis. **27 Suppl 1**:S12-S20.

150. **Main-Hester, K. L., K. M. Colpitts, G. A. Thomas, F. C. Fang, and S. J. Libby**. 2008. Coordinate regulation of *Salmonella* pathogenicity island 1 (SPI1) and SPI4 in *Salmonella enterica* serovar Typhimurium. Infect. Immun. **76**:1024-1035.

151. **Majtan, T., L. Majtanova, J. Timko, and V. Majtan**. 2007. Oligonucleotide microarray for molecular characterization and genotyping of *Salmonella spp.* strains. J. Antimicrob. Chemother. **60**:937-946.

152. **Malorny, B., C. Bunge, B. Guerra, S. Prietz, and R. Helmuth**. 2007. Molecular characterisation of *Salmonella* strains by an oligonucleotide multiprobe microarray. Mol. Cell Probes **21**:56-65.

153. **Malorny, B., E. Paccassoni, P. Fach, C. Bunge, A. Martin, and R. Helmuth**. 2004. Diagnostic real-time PCR for the detection of *Salmonella* in food. Appl. Environ. Microbiol. **70**:7046-7052.

154. **Marcus, S. L., J. H. Brumell, C. G. Pfeifer, and B. B. Finlay**. 2000. *Salmonella* pathogenicity islands: big virulence in small packages. Microbes. Infect. **2**:145-156.

155. **Mazel, D., B. Dychinco, V. A. Webb, and J. Davies**. 2000. Antibiotic resistance in the ECOR collection: integrons and identification of a novel *aad* gene. Antimicrob. Agents Chemother. **44**:1568-1574.

156. **McClelland, M., J. Parkhill, and E. Morgan**. 2007. *Salmonella* Pathogenicity Islands, p. 67-88. *In* M. Rhen, D. Maskell, P. Mastroeni, and J. Threlfall (eds.), *Salmonella* molecular biology and pathogenesis. Horizon Bioscience.

157. **McClelland, M., K. E. Sanderson, J. Spieth, S. W. Clifton, P. Latreille, L. Courtney, S. Porwollik, J. Ali, M. Dante, F. Du, S. Hou, D. Layman, S. Leonard, C. Nguyen, K. Scott, A. Holmes, N. Grewal, E. Mulvaney, E. Ryan, H. Sun, L. Florea, W. Miller, T. Stoneking, M. Nhan, R. Waterston, and R. K. Wilson**. 2001. Complete genome sequence of *Salmonella enterica* serovar Typhimurium LT2. Nature **413**:852-856.

158. **McGhie, E. J., R. D. Hayward, and V. Koronakis**. 2001. Cooperation between actin-binding proteins of invasive *Salmonella*: SipA potentiates SipC nucleation and bundling of actin. EMBO J. **20**:2131-2139.

159. **McNabb, S. J., R. A. Jajosky, P. A. Hall-Baker, D. A. Adams, P. Sharp, C. Worshams, W. J. Anderson, A. J. Javier, G. J. Jones, D. A. Nitschke, A. Rey, and M. S. Wodajo**. 2008. Summary of notifiable diseases--United States, 2006. MMWR Morb. Mortal. Wkly.Rep. **55**:1-92.

160. **McQuiston, J. R., P. I. Fields, R. V. Tauxe, and J. M. Logsdon, Jr.** 2008. Do *Salmonella* carry spare tyres? Trends Microbiol. **16**:142-148.

161. **Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe**. 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. **5**:607-625.

162. **Meunier, D., D. Boyd, M. R. Mulvey, S. Baucheron, C. Mammina, A. Nastasi, E. Chaslus-Dancla, and A. Cloeckaert**. 2002. Identification of *Salmonella enterica* serovar Typhimurium DT104 antibiotic resistance genomic island I in serotype Paratyphi B. Emerg. Infect. Dis. **8**:430-433.

163. **Miao, E. A. and S. I. Miller**. 2000. A conserved amino acid sequence directing intracellular type III secretion by *Salmonella typhimurium*. Proc. Natl. Acad. Sci. U.S.A **97**:7539-7544.

164. **Miko, A., B. Guerra, A. Schroeter, C. Dorn, and R. Helmuth**. 2002. Molecular charcaterization of multiresistent d-tartrate-positive *Salmonella enterica* serovar Paratyphi B isolates. J. Clin. Microbiol. **40**:3184-3191.

165. **Miko, A., K. Pries, A. Schroeter, and R. Helmuth**. 2003. Multiple-drug resistance in D-tartrate-positive *Salmonella enterica* serovar Paratyphi B isolates from poultry is mediated by class 2 integrons inserted into the bacterial chromosome. Antimicrob. Agents Chemother. **47**:3640-3643.

166. **Mirold, S., W. Rabsch, M. Rohde, S. Stender, H. Tschape, H. Russmann, E. Igwe, and W. D. Hardt**. 1999. Isolation of a temperate bacteriophage encoding the type III effector protein SopE from an epidemic *Salmonella typhimurium* strain. Proc. Natl. Acad. Sci. U.S.A. **96**:9845-9850.

167. **Mmolawa PT, S. H. H. MW.** 2003. Bacteriophage ST64B, a genetic mosaic of genes from diverse sources isolated from *Salmonella enterica* serovar Typhimurium DT 64. J. Bacteriol. **185**:6481-6485.

168. **Mullis, K. B. and F. A. Faloona**. 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol. **155**:335-350.

169. **Mulvey, M. R., D. Boyd, A. Cloeckaert, R. Ahmed, and L. K. Ng**. 2004. Emergence of multidrug-resistant *Salmonella* Paratyphi B dT+, Canada. Emerg. Infect. Dis. **10**:1307-1310.

170. **Mushegian, A. R. and E. V. Koonin**. 1996. A minimal gene set for cellular life derived by comparison of complete bacterial genomes. Proc. Natl. Acad. Sci. U.S.A **93**:10268-10273.

171. **Nair, S., D. House, A. Bishop, and J. Wain**. 2006. Invasive salmonellosis in humans. 5 January 2006, posting date, *In* A. Böck, R. Curtiss III, J. B. Kaper, F. C. Neidhardt, T. Nyström, K. E. Rudd, and C. L. Squires (eds.), EcoSal-*Escherichia coli* and *Salmonella*: cellular and molecular biology. [Online.] http://www.ecosal.org. ASM Press, Washington, D.C.

172. **Naumann, T. A. and W. S. Reznikoff**. 2002. Tn*5* transposase with an altered specificity for transposon ends. J. Bacteriol. **184**:233-240.

173. **Navarre, W. W., T. A. Halsey, D. Walthers, J. Frye, M. McClelland, J. L. Potter, L. J. Kenney, J. S. Gunn, F. C. Fang, and S. J. Libby**. 2005. Co-regulation of *Salmonella enterica* genes required for virulence and resistance to antimicrobial peptides by SlyA and PhoP/PhoQ. Mol. Microbiol. **56**:492-508.

174. **Ng, L. K., I. Martin, M. Alfa, and M. Mulvey**. 2001. Multiplex PCR for the detection of tetracycline resistant genes. Mol. Cell Probes **15**:209-215.

175. **Ng, L. K., M. R. Mulvey, I. Martin, G. A. Peters, and W. Johnson**. 1999. Genetic characterization of antimicrobial resistance in Canadian isolates of *Salmonella* serovar Typhimurium DT104. Antimicrob. Agents Chemother. **43**:3018-3021.

176. **Nield, B. S., A. J. Holmes, M. R. Gillings, G. D. Recchia, B. C. Mabbutt, K. M. Nevalainen, and H. W. Stokes**. 2001. Recovery of new integron classes from environmental DNA. FEMS Microbiol. Lett. **195**:59-65.

177. **Nordmann, P.** 1998. Trends in beta-lactam resistance among Enterobacteriaceae. Clin. Infect. Dis. **27 Suppl 1**:S100-S106.

178. **Olive, D. M. and P. Bean**. 1999. Principles and applications of methods for DNA-based typing of microbial organisms. J. Clin. Microbiol. **37**:1661-1669.

179. **Pardon, P., R. Sanchis, J. Marly, F. Lantier, L. Guilloteau, D. Buzoni-Gatel, I. P. Oswald, M. Pepin, B. Kaeffer, P. Berthon, and .** 1990. Experimental ovine salmonellosis (*Salmonella abortusovis*): pathogenesis and vaccination. Res. Microbiol. **141**:945-953.

180. **Parkhill, J., G. Dougan, K. D. James, N. R. Thomson, D. Pickard, J. Wain, C. Churcher, K. L. Mungall, S. D. Bentley, M. T. Holden, M. Sebaihia, S. Baker, D.**

Basham, K. Brooks, T. Chillingworth, P. Connerton, A. Cronin, P. Davis, R. M. Davies, L. Dowd, N. White, J. Farrar, T. Feltwell, N. Hamlin, A. Haque, T. T. Hien, S. Holroyd, K. Jagels, A. Krogh, T. S. Larsen, S. Leather, S. Moule, P. O'Gaora, C. Parry, M. Quail, K. Rutherford, M. Simmonds, J. Skelton, K. Stevens, S. Whitehead, and B. G. Barrell. 2001. Complete genome sequence of a multiple drug resistant *Salmonella enterica* serovar Typhi CT18. Nature **413**:848-852.

181. **Pegues, D. A., M. J. Hantman, I. Behlau, and S. I. Miller**. 1995. PhoP/PhoQ transcriptional repression of *Salmonella typhimurium* invasion genes: evidence for a role in protein secretion. Mol. Microbiol. **17**:169-181.

182. **Pelludat, C., R. Prager, H. Tschäpe, W. Rabsch, J. Schuchhardt, and W. D. Hardt**. 2005. Pilot study to evaluate microarray hybridization as a tool for *Salmonella enterica* serovar Typhimurium strain differentiation. J. Clin. Microbiol. **43**:4092-4106.

183. **Perreten, V. and P. Boerlin**. 2003. A new sulfonamide resistance gene (*sul3*) in *Escherichia coli* is widespread in the pig population of Switzerland. Antimicrob. Agents Chemother. **47**:1169-1172.

184. **Peters, T. M., C. Maguire, E. J. Threlfall, I. S. Fisher, N. Gill, and A. J. Gatto**. 2003. The Salm-gene project - a European collaboration for DNA fingerprinting for Food-related salmonellosis. Euro. Surveill. **8**:46-50.

185. **Pickard, D., J. Wain, S. Baker, A. Line, S. Chohan, M. Fookes, A. Barron, P. O. Gaora, J. A. Chabalgoity, N. Thanky, C. Scholes, N. Thomson, M. Quail, J. Parkhill, and G. Dougan**. 2003. Composition, acquisition, and distribution of the Vi exopolysaccharide-encoding *Salmonella enterica* pathogenicity island SPI-7. J. Bacteriol. **185**:5055-5065.

186. **Popoff, M. Y., J. Bockemühl, F. W. Brenner, and L. L. Gheesling**. 2001. Supplement 2000 (no. 44) to the Kauffmann-White scheme. Res. Microbiol. **152**:907-909.

187. **Popoff, M. Y. and L. Le Minor**. 2001. Antigenic formulas of the *Salmonella* serovars, 8th revision. WHO Collaborating Centre for Reference and Research on Salmonella, Institut Pasteur, Paris, France.

188. **Porwollik, S., E. F. Boyd, C. Choy, P. Cheng, L. Florea, E. Proctor, and M. McClelland**. 2004. Characterization of *Salmonella enterica* subspecies I genovars by use of microarrays. J. Bacteriol. **186**:5883-5898.

189. **Porwollik, S. and M. McClelland**. 2003. Lateral gene transfer in *Salmonella*. Microbes Infect. **5**:977-989.

190. **Porwollik, S., R. M. Wong, and M. McClelland**. 2002. Evolutionary genomics of *Salmonella*: gene acquisitions revealed by microarray analysis. Proc. Natl. Acad. Sci. U.S.A **99**:8956-8961.

191. **Prager, R., A. Liesegang, W. Rabsch, B. Gericke, W. Thiel, W. Voigt, R. Helmuth, L. Ward, and H. Tschape**. 1999. Clonal relationship of *Salmonella enterica* serovar Typhimurium phage type DT104 in Germany and Austria. Zentralbl. Bakteriol. **289**:399-414.

192. **Prager, R., W. Rabsch, W. Streckel, W. Voigt, E. Tietze, and H. Tschäpe**. 2003. Molecular properties of *Salmonella enterica* serotype Paratyphi B distinguish between its systemic and its enteric pathovars. J. Clin. Microbiol. **41**:4270-4278.

193. **Rabsch, W.** 2007. *Salmonella typhimurium* phage typing for pathogens. Methods Mol. Biol. **394**:177-211.

194. **Rahn, K., S. A. De Grandis, R. C. Clarke, S. A. McEwen, J. E. Galán, C. Ginocchio, R. Curtiss, III, and C. L. Gyles**. 1992. Amplification of an *invA* gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*. Mol. Cell. Probes **6**:271-279.

195. **Randall, L. P., S. W. Cooles, M. K. Osborn, L. J. Piddock, and M. J. Woodward**. 2004. Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of *Salmonella enterica* isolated from humans and animals in the UK. J. Antimicrob. Chemother. **53**:208-216.

196. **Raupach, B., N. Kurth, K. Pfeffer, and S. H. Kaufmann**. 2003. *Salmonella typhimurium* strains carrying independent mutations display similar virulence phenotypes yet are controlled by distinct host defense mechanisms. J. Immunol. **170**:6133-6140.

197. **Reen, F. J., E. F. Boyd, S. Porwollik, B. P. Murphy, D. Gilroy, S. Fanning, and M. McClelland**. 2005. Genomic comparisons of *Salmonella enterica* serovar Dublin, Agona, and Typhimurium strains recently isolated from milk filters and bovine samples from Ireland, using a *Salmonella* microarray. Appl. Environ. Microbiol. **71**:1616-1625.

198. **Reeves, P.** 1993. Evolution of *Salmonella* O antigen variation by interspecific gene transfer on a large scale. Trends Genet. **9**:17-22.

199. **Rhen, M., D. Maskell, P. Mastroeni, and J. Threlfall**. 2007. *Salmonella*: Molecular Biology and Pathogenesis. Horizon Bioscience.

200. **Ribot, E. M., M. A. Fair, R. Gautom, D. N. Cameron, S. B. Hunter, B. Swaminathan, and T. J. Barrett**. 2006. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. Foodborne. Pathog. Dis. **3**:59-67.

201. **Robicsek, A., G. A. Jacoby, and D. C. Hooper**. 2006. The worldwide emergence of plasmid-mediated quinolone resistance. Lancet Infect. Dis. **6**:629-640.

202. **Rodriguez, I., M. C. Martin, M. C. Mendoza, and M. R. Rodicio**. 2006. Class 1 and class 2 integrons in non-prevalent serovars of *Salmonella enterica*: structure and association with transposons and plasmids. J. Antimicrob. Chemother. **58**:1124-1132.

203. **Romeo, T. and J. L. Snoep**. 2005. Glycolysis and flux control, posted october 7, 2005, *In* A. Böck, R. Curtiss III, J. B. Kaper, F. C. Neidhardt, T. Nyström, K. E. Rudd, and C. L. Squires (eds.), EcoSal-*Escherichia coli* and *Salmonella*: cellular and molecular biology. [Online.] http://www.ecosal.org. ASM Press, Washington, D.C.

204. **Rosenstraus, M., Z. Wang, S.-Y. Chang, D. DeBonville, and J. P. Spadoro**. 1998. An internal control for routine diagnostic PCR: design, properties, and effect on clinical performance. J. Clin. Microbiol. **36**:191-197.

205. **Rowe-Magnus, D. A. and D. Mazel**. 1999. Resistance gene capture. Curr. Opin. Microbiol. **2**:483-488.

206. **Sachse, K., H. Hotzel, P. Slickers, T. Ellinger, and R. Ehricht**. 2005. DNA microarray-based detection and identification of *Chlamydia* and C*hlamydophila* spp. Mol. Cell Probes **19**:41-50.

207. **Salmon, D. E.** 1884. The discovery of the germ of swine-plague. Science **3**:155-158.

208. **Salyers, A. A. and N. B. Shoemaker**. 1997. Conjugative transposons. Genet. Eng (N.Y.) **19**:89-100.

209. **Sandvang, D., F. M. Aarestrup, and L. B. Jensen**. 1997. Characterisation of integrons and antibiotic resistance genes in Danish multiresistant *Salmonella enterica* Typhimurium DT104. FEMS Microbiol. Lett. **157**:177-181.

210. **Sartor, M., J. Schwanekamp, D. Halbleib, I. Mohamed, S. Karyala, M. Medvedovic, and C. R. Tomlinson**. 2004. Microarray results improve significantly as hybridization approaches equilibrium. Biotechniques **36**:790-796.

211. **Sawer, R. G. and D. P. Clark**. 2008. Fermentative pyruvate and acetyl-coenzyme A metabolism, posted Jul 27, 2004, *In* A. Böck, R. Curtiss III, J. B. Kaper, P. D. Karp, F. C. Neidhardt, T. Nyström, J. M. Slauch, and C. L. Squires (eds.), EcoSal-*Escherichia coli* and *Salmonella*: cellular and molecular biology. [Online.] http://www.ecosal.org. ASM Press, Washington D.C.

212. **Schlumberger, M. C. and W. D. Hardt**. 2006. *Salmonella* type III secretion effectors: pulling the host cell's strings. Curr. Opin. Microbiol. **9**:46-54.

213. **Schwartz E, K. M. R. B.** 1988. IS150: distribution, nucleotide sequence and phylogenetic relationships of a new *E. coli* insertion element. Nucl. Acids Res. **16**:6789-6802.

214. **Schwartz, D. C. and C. R. Cantor**. 1984. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. Cell **37**:67-75.

215. **Schwartz, D. C., W. Saffran, J. Welsh, R. Haas, M. Goldenberg, and C. R. Cantor**. 1983. New techniques for purifying large DNAs and studying their properties and packaging. Cold Spring Harb. Symp. Quant. Biol. **47 Pt 1**:189-195.

216. **Schwarz, S. and E. Chaslus-Dancla**. 2001. Use of antimicrobials in veterinary medicine and mechanisms of resistance. Vet. Res. **32**:201-225.

217. **Sefton, A. M.** 2002. Mechanisms of antimicrobial resistance: their clinical relevance in the new millennium. Drugs **62**:557-566.

218. **Siebold, C., K. Flukiger, R. Beutler, and B. Erni**. 2001. Carbohydrate transporters of the bacterial phosphoenolpyruvate: sugar phosphotransferase system (PTS). FEBS Lett. **504**:104-111.

219. **Silverman, M., J. Zieg, M. Hilmen, and M. Simon**. 1979. Phase variation in *Salmonella*: genetic analysis of a recombinational switch. Proc. Natl. Acad. Sci. U.S.A **76**:391-395.

220. **Skov, M. N., J. J. Madsen, C. Rahbek, J. Lodal, J. B. Jespersen, J. C. Jorgensen, H. H. Dietz, M. Chriel, and D. L. Baggesen**. 2008. Transmission of *Salmonella* between wildlife and meat-production animals in Denmark. J. Appl. Microbiol. **105**:1558-1568.

221. **Slauch, J. M., A. A. Lee, M. J. Mahan, and J. J. Mekalanos**. 1996. Molecular characterization of the *oafA* locus responsible for acetylation of *Salmonella typhimurium* O-antigen: *oafA* is a member of a family of integral membrane trans-acylases. J. Bacteriol. **178**:5904-5909.

222. **Slauch, J. M., M. J. Mahan, P. Michetti, M. R. Neutra, and J. J. Mekalanos**. 1995. Acetylation (O-factor 5) affects the structural and immunological properties of *Salmonella typhimurium* lipopolysaccharide O antigen. Infect. Immun. **63**:437-441.

223. **Smith, N. H., P. Beltran, and R. K. Selander**. 1990. Recombination of *Salmonella* phase 1 flagellin genes generates new serovars. J. Bacteriol. **172**:2209-2216.

224. **Snavely, M. D., S. A. Gravina, T. T. Cheung, C. G. Miller, and M. E. Maguire**. 1991. Magnesium transport in *Salmonella typhimurium*. Regulation of *mgtA* and *mgtB* expression. J. Biol. Chem. **266**:824-829.

225. **Southern, E., K. Mir, and M. Shchepinov**. 1999. Molecular interactions on microarrays. Nat. Genet. **21**:5-9.

226. **Stanley, T. L., C. D. Ellermeier, and J. M. Slauch**. 2000. Tissue-specific gene expression identifies a gene in the lysogenic phage Gifsy-1 that affects *Salmonella enterica* serovar Typhimurium survival in Peyer's patches. J. Bacteriol. **182**:4406-4413.

227. **Steen, R. and O. Skold**. 1985. Plasmid-borne or chromosomally mediated resistance by Tn*7* is the most common response to ubiquitous use of trimethoprim. Antimicrob. Agents Chemother. **27**:933-937.

228. **Stratton, J., L. Stefaniw, K. Grimsrud, D. H. Werker, A. Ellis, E. Ashton, L. Chui, E. Blewett, R. Ahmed, C. Clark, F. Rodgers, L. Trottier, and B. Jensen**. 2001. Outbreak of *Salmonella paratyphi* B var *java* due to contaminated alfalfa sprouts in Alberta, British Columbia and Saskatchewan. Can. Commun. Dis. Rep. **27**:133-137.

229. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan**. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33**:2233-2239.

230. **Teplitski, M., R. I. Goodier, and B. M. Ahmer**. 2003. Pathways leading from BarA/SirA to motility and virulence gene expression in *Salmonella*. J. Bacteriol. **185**:7257-7265.

231. **Thankavel, K., A. H. Shah, M. S. Cohen, T. Ikeda, R. G. Lorenz, R. Curtiss, III, and S. N. Abraham**. 1999. Molecular basis for the enterocyte tropism exhibited by *Salmonella* typhimurium type 1 fimbriae. J. Biol. Chem. **274**:5797-5809.

232. **Thomas, C. M. and K. M. Nielsen**. 2005. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nat. Rev. Microbiol. **3**:711-721.

233. **Thomson, N. R., D. J. Clayton, D. Windhorst, G. Vernikos, S. Davidson, C. Churcher, M. A. Quail, M. Stevens, M. A. Jones, M. Watson, A. Barron, A. Layton, D. Pickard, R. A. Kingsley, A. Bignell, L. Clark, B. Harris, D. Ormond, Z. Abdellah, K. Brooks, I. Cherevach, T. Chillingworth, J. Woodward, H. Norberczak, A. Lord, C. Arrowsmith, K. Jagels, S. Moule, K. Mungall, M. Sanders, S. Whitehead, J. A. Chabalgoity, D. Maskell, T. Humphrey, M. Roberts, P. A. Barrow, G. Dougan, and J. Parkhill**. 2008. Comparative genome analysis of *Salmonella* Enteritidis PT4 and *Salmonella* Gallinarum 287/91 provides insights into evolutionary and host adaptation pathways. Genome Res. **18**:1624-1637.

234. **Thorns, C. J.** 2000. Bacterial food-borne zoonoses. Rev. Sci. Tech. **19**:226-239.

235. **Threlfall, E. J.** 2002. Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food- and water-borne infections. FEMS Microbiol. Rev. **26**:141-148.

236. **Threlfall, E. J., J. A. Frost, L. R. Ward, and B. Rowe**. 1994. Epidemic in cattle and humans of *Salmonella typhimurium* DT 104 with chromosomally integrated multiple drug resistance. Vet. Rec. **134**:577.

237. **Threlfall, J., B. Levent, K. L. Hopkins, E. de Pinna, L. R. Ward, and D. J. Brown**. 2005. Multidrug-resistant *Salmonella* Java. Emerg. Infect. Dis. **11**:170-171.

238. **Toleman, M. A., P. M. Bennett, and T. R. Walsh**. 2006. *ISCR* elements: Novel gene-capturing systems of the 21st century? Microbiol. Mol. Biol. Rev. **70**:296-316.

239. **Uchiya, K., M. A. Barbieri, K. Funato, A. H. Shah, P. D. Stahl, and E. A. Groisman**. 1999. A *Salmonella* virulence protein that inhibits cellular trafficking. EMBO J. **18**:3924-3933.

240. **van der Klooster, J. M. and H. J. Roelofs**. 1997. Management of *Salmonella* infections during pregnancy and puerperium. Neth. J. Med. **51**:83-86.

241. **van Hoek, A. H., I. M. Scholtens, A. Cloeckaert, and H. J. Aarts**. 2005. Detection of antibiotic resistance genes in different *Salmonella* serovars by oligonucleotide microarray analysis. J. Microbiol. Methods **62**:13-23.

242. **Van Immerseel, F., L. Meulemans, J. De Buck, F. Pasmans, P. Velge, E. Bottreau, F. Haesebrouck, and R. Ducatelle**. 2004. Bacteria-host interactions of *Salmonella* Paratyphi B dT+ in poultry. Epidemiol. Infect. **132**:239-243.

243. **van Pelt, W., H. van der Zee, W. J. B. Wannet, A. W. van de Giessen, D. J. Mevius, N. M. Bolder, R. E. Komijn, and Y. T. H. P. van Duynhoven**. 2003. Explosive increase of *Salmonella* Java in poultry in the Netherlands: Consequences for public health. Eurosurveilance **8**:31-35.

244. **van, d. V., A. J. Bäumler, R. M. Tsolis, and F. Heffron**. 1998. Multiple fimbrial adhesins are required for full virulence of *Salmonella typhimurium* in mice. Infect. Immun. **66**:2803-2808.

245. **Vander, B. C. and A. M. Kropinski**. 2000. Sequence of the genome of *Salmonella* bacteriophage P22. J. Bacteriol. **182**:6472-6481.

246. **Vazquez-Torres, A., J. Jones-Carson, A. J. Bäumler, S. Falkow, R. Valdivia, W. Brown, M. Le, R. Berggren, W. T. Parks, and F. C. Fang**. 1999. Extraintestinal dissemination of *Salmonella* by CD18-expressing phagocytes. Nature **401**:804-808.

247. **Vernikos, G. S. and J. Parkhill**. 2006. Interpolated variable order motifs for identification of horizontally acquired DNA: revisiting the *Salmonella* pathogenicity islands. Bioinformatics. **22**:2196-2203.

248. **Vishnubhatla, A., D. Y. Fung, R. D. Oberst, M. P. Hays, T. G. Nagaraja, and S. J. Flood**. 2000. Rapid 5' nuclease (TaqMan) assay for detection of virulent strains of *Yersinia enterocolitica*. Appl. Environ. Microbiol. **66**:4131-4135.

249. **Voetsch, A. C., T. J. Van Gilder, F. J. Angulo, M. M. Farley, S. Shallow, R. Marcus, P. R. Cieslak, V. C. Deneen, and R. V. Tauxe**. 2004. FoodNet estimate of the burden of illness caused by nontyphoidal *Salmonella* infections in the United States. Clin. Infect. Dis. **38 Suppl 3**:S127-S134.

250. **Wallis, T. S. and P. A. Barrow**. 2005. *Salmonella* epidemiology and pathogenesis in food-producing animals, posted july 25, 2005, *In* A. Böck, R. Curtiss III, J. B. Kaper, F. C. Neidhardt, T. Nyström, K. E. Rudd, and C. L. Squires (eds.), EcoSal-*Escherichia coli* and *Salmonella*: cellular and molecular biology. [Online.] http://www.ecosal.org. ASM Press, Washington, D.C.

251. **Wallis, T. S., S. M. Paulin, J. S. Plested, P. R. Watson, and P. W. Jones**. 1995. The *Salmonella dublin* virulence plasmid mediates systemic but not enteric phases of salmonellosis in cattle. Infect. Immun. **63**:2755-2761.

252. **Wang, L., K. Andrianopoulos, D. Liu, M. Y. Popoff, and P. R. Reeves**. 2002. Extensive variation in the O-antigen gene cluster within one *Salmonella enterica* serogroup reveals an unexpected complex history. J. Bacteriol. **184**:1669-1677.

253. **Ward, L. R., J. D. H. de Sa, and B. Rowe**. 1987. A phage-typing scheme for *Salmonella enteritidis*. Epidemiol. Infect. **99**:291-294.

254. **Ward, L. R., J. Threlfall, H. R. Smith, and S. J. O'Brien**. 2000. *Salmonella enteritidis* epidemic. Science **287**:1753-1754.

255. **Wattiau, P., T. Weijers, P. Andreoli, C. Schliker, H. V. Veken, H. M. Maas, A. J. Verbruggen, M. E. Heck, W. J. Wannet, H. Imberechts, and P. Vos**. 2008. Evaluation of the Premi(R)Test *Salmonella*, a commercial low-density DNA microarray system intended for routine identification and typing of *Salmonella enterica*. Int. J. Food Microbiol. **123**:293-298.

256. **Weening, E. H., J. D. Barker, M. C. Laarakker, A. D. Humphries, R. M. Tsolis, and A. J. Bäumler**. 2005. The *Salmonella enterica* serotype Typhimurium *lpf, bcf,*

stb, *stc*, *std*, and *sth* fimbrial operons are required for intestinal persistence in mice. Infect. Immun. **73**:3358-3366.

257. **Weill, F. X., L. Fabre, B. Grandry, P. A. Grimont, and I. Casin**. 2005. Multiple-antibiotic resistance in *Salmonella enterica* serotype Paratyphi B isolates collected in France between 2000 and 2003 is due mainly to strains harboring *Salmonella* genomic islands 1, 1-B, and 1-C. Antimicrob. Agents Chemother. **49**:2793-2801.

258. **White, P. A., C. J. McIver, and W. D. Rawlinson**. 2001. Integrons and gene cassettes in the enterobacteriaceae. Antimicrob. Agents Chemother. **45**:2658-2661.

259. **Wilson, J. A. and P. A. Gulig**. 1998. Regulation of the *spvR* gene of the *Salmonella typhimurium* virulence plasmid during exponential-phase growth in intracellular salts medium and at stationary phase in L broth. Microbiology **144 (Pt 7)**:1823-1833.

260. **Wong, K. K., M. McClelland, L. C. Stillwell, E. C. Sisk, S. J. Thurston, and J. D. Saffer**. 1998. Identification and sequence analysis of a 27-kilobase chromosomal fragment containing a *Salmonella* pathogenicity island located at 92 minutes on the chromosome map of *Salmonella enterica* serovar Typhimurium LT2. Infect. Immun. **66**:3365-3371.

261. **Wood, M. W., M. A. Jones, P. R. Watson, S. Hedges, T. S. Wallis, and E. E. Galyov**. 1998. Identification of a pathogenicity island required for *Salmonella* enteropathogenicity. Mol. Microbiol. **29**:883-891.

262. **Woodward, M. J., I. McLaren, and C. Wray**. 1989. Distribution of virulence plasmids within *Salmonellae*. J. Gen. Microbiol. **135**:503-511.

263. **World Health Organization**. 2006. WHO Global Salm-Surv: Progress Report 2000-2005. Available at http://www.who.int/salmsurv/links/GSSProgressReport2005.pdf.

264. **Wu, K. Y., G. R. Liu, W. Q. Liu, A. Q. Wang, S. Zhan, K. E. Sanderson, R. N. Johnston, and S. L. Liu**. 2005. The genome of *Salmonella enterica* serovar Gallinarum: distinct insertions/deletions and rare rearrangements. J. Bacteriol. **187**:4720-4727.

265. **Xiang, S. H., A. M. Haase, and P. R. Reeves**. 1993. Variation of the *rfb* gene clusters in *Salmonella enterica*. J. Bacteriol. **175**:4877-4884.

266. **Yoshida, C., K. Franklin, P. Konczy, J. R. McQuiston, P. I. Fields, J. H. Nash, E. N. Taboada, and K. Rahn**. 2007. Methodologies towards the development of an oligonucleotide microarray for determination of *Salmonella* serotypes. J. Microbiol. Methods **70**:261-271.

267. **Zhao, S., S. Qaiyumi, S. Friedman, R. Singh, S. L. Foley, D. G. White, P. F. McDermott, T. Donkar, C. Bolin, S. Munro, E. J. Baron, and R. D. Walker**. 2003. Characterization of *Salmonella enterica* serotype Newport isolated from humans and food animals. J. Clin. Microbiol. **41**:5366-5371.

268. **Zhou, D., L. M. Chen, L. Hernandez, S. B. Shears, and J. E. Galan**. 2001. A *Salmonella* inositol polyphosphatase acts in conjunction with other bacterial effectors to promote host cell actin cytoskeleton rearrangements and bacterial internalization. Mol. Microbiol. **39**:248-259.

269. **Zieg, J., M. Hilmen, and M. Simon**. 1978. Regulation of gene expression by site-specific inversion. Cell **15**:237-244.

270. **Zieg, J., M. Silverman, M. Hilmen, and M. Simon**. 1977. Recombinational switch for gene expression. Science **196**:170-172.

6. Eidesstattliche Erklärung

Hiermit erkläre ich, die vorliegende Arbeit selbständig verfasst und alle Versuche eigenständig durchgeführt zu haben. Es wurden keine anderen Hilfsmittel als die Angegebenen verwendet. Diese Arbeit hat weder in gleicher, noch in ähnlicher Form einem anderen Prüfungsverfahren vorgelegen.

Berlin, _____ _____

Stephan Hühn

7. List of publications

Parts of this work were already published at following journals:

Huehn S and Malorny B, DNA Microarray for molecular epidemiology of *Salmonella,* Methods Mol Biol, 2009;551:249-85, ISBN 978-1-60327-998-7

Huehn S, Bunge C, Junker E, Helmuth R, Malorny B, Poultry associated *Salmonella enterica* subsp. *enterica* serovar 4,12:d:- reveals high clonality and a distinct pathogenicity gene repertoire,
Appl Environ Microbiol, 2009 Feb;75(4):1011-20. (PMID: 19114530)

Huehn S, Helmuth R, Bunge C, Guerra B, Junker E, Davies R, Wattiau P, van Pelt W, Malorny B, Characterization of pathogenic and resistant genome repertoire reveals two clonal lines in *Salmonella enterica* subsp. *enterica* serovar Paratyphi B d-tartrate positive,
Foodborne Pathog Dis, 2009 May;6(4):431-43. (PMID: 19292689)

Hauser E, **Hühn S**, Junker E, Jaber M, Schroeter A, Helmuth R, Rabsch W, Winterhoff N, Malorny B, [Characterisation of a phenotypic monophasic variant belonging to *Salmonella enterica* subsp. *enterica* serovar Typhimurium from wild birds and its possible transmission to cats and humans],
Berl Münch Tierärztl Wochenschr. 2009 May-Jun;122(5-6):169-77. German.
(PMID: 19517930)

Huehn S, La Ragione RM, Anjum M, Saunders M, Woodward MJ, Bunge C, Helmuth R, Hauser E, Guerra B, Beutlich J, Brisabois A, Peters T, Svensson L, Madajczak G, Litrup E, Imre A, Herrera-Leon S, Mevius D, Newell DG, Malorny B, Virulo- and Resistance Typing of *Salmonella enterica* serovars relevant to human health in Europe,
Foodborne Pathog Dis, in press

Conference proceedings

Posters

DNA microarray for the characterization and typing of *Salmonella*: A new tool for risk analysis, EU-US Priority Setting of Food borne and Zoonotic Pathogens, 19. - 21.06.2006, Berlin, Germany.

DNA microarray for the characterization and typing of *Salmonella*: A new tool for risk analysis, MED-VET-NET WP26 Virulotyping Meeting, ISS, 01. - 02.03.2007, Rome, Italy.

Work package WP26 annual report and, 3rd annual MED-VET-NET Meeting, 27.06.-29.06.2007, Lucca, Italy.

Virulotyping of *Salmonella* isolates positive for Genomic Island 1, 3rd annual MED-VET-NET Meeting, 27.06.-29.06.2007, Lucca, Italy.

The development of a microarray procedure for the detection and characterization of *Salmonella,* 1st BIOTRACER General Meeting, 14.-16.11.2007, Athens, Greece.

Results on comparative virulotyping analysis of a subset of *Salmonella* across partner institutes in Europe, 4th annual MED-VET-NET Meeting, 11.06.-14.06.2007, Saint Malo, France.

DNA microarray for the characterization and typing of *Salmonella enterica* subsp. *enterica*: A novel tool for risk analysis, 3rd FEMS Kongress 2009, 28.06.-02.07.2009, Göteborg, Sweden.

Talks

The BfR Microarray – PhD external meeting, Biotracer General Kick-off Meeting, Vienna, Austria, 18.-21.02.2007

A microarray for the characterization and typing of *Salmonella* isolates: an update, MED-VET-NET WP26 Virulotyping Meeting, ISS, Rome, Italy, 01. - 02.03.2007

DNA Microarrays zur Charakterisierung und Typisierung von *Salmonella* Isolaten aus Lebensmitteln, 9. Fachsymposium Lebensmittelmikrobiologie der DGHM/VAAM, Kloster Seeon, Germany, 21.03.-23.03.2007

DNA Microarray for characterisation and typing of an emerging *Salmonella* serovar 4,12:d:- in Germany, 3rd annual MED-VET-NET Meeting, Lucca, Italy**,** 27.06.-29.06.2007

Charakterisierung von Salmonellen mit DNA Microarrays – Ein neues Tool zur Gefahrenidentifizierung, BfR Seminar, Berlin, Germany, 04.07.2007

***Salmonella* and DNA Microarrays,** Workshop PathogenCombat, CONGEN Biotechnologie GmbH, Berlin, Germany, 29.10.2007

PhD meeting Athens: BfR research activities, 1st BIOTRACER General Meeting, Athens, Greece, 14.-16-11.2007

Results of microarray analysis from selected Virulotyping *Salmonella* strains, MED-VET-NET WP26 Meeting, Berlin, Germany, 26.-27.11.2007

Genotypische Charakterisierung von *Salmonella* 4,12:d:-, ein Serovar mit hoher Prävalenz in Masthähnchen, Fachsymposium Lebensmittelmikrobiologie der DGHM/VAAM, Stuttgart, Germany, 09.-11.04.2008 10

Huehn S., Bunge C., Helmuth R. and B. Malorny, 2008
IP 03 – Hazard identification of *Salmonella* in the food chain, 2nd status meeting of BMBF project: Food-borne zoonotic Infections of humans (FBI-Zoo), Münster, Germany, 20.-21.05.2008

Huehn S., Bunge C., Helmuth R. and B. Malorny, 2008
DNA Chip-technology: A new method for fast characterization of zoonotic pathogens like *Salmonella*, Free University of Berlin, biochemistry branch, Berlin, Germany, 04.07.2008

Huehn S. and B. Malorny, 2008
A DNA microarray for Characterisation and typing of *Salmonella* Isolates, PhD Meeting, DTU, Kopenhagen, Denmark, 17.10.2008

Huehn S. and B. Malorny, 2008
Development and validation of a DNA microarray for Characterisation and typing of *Salmonella* Isolates, Workshop PathogenCombat, CONGEN Biotechnologie GmbH, Berlin, Germany, 24.10.2008

Huehn S., WP26 participants, Reiner Helmuth and B. Malorny, 2009
Microarray Virulotyping of European *Salmonella* Isolates, MED-VET-NET WP26 final scientific meeting, Veterinary Laboratories Agency, Weybridge, United Kingdom, 16.01.2009

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9. Curriculum vitae

For reasons of data protection,
the curriculum vitae is not included in the online version

10. Appendix I

Table A1: List of PCR primer sequences for the detection of the presence of genes in *Salmonella enterica* subspecies *enterica* serovars.

Gene	Accessionno.	Forwardprimer	Backwardprimer	Size in bp	Reference
aac(3)_IV	X01385	gttacaccggaccttgga	aacggcattgagcgtcag	674	(51)
aacC1	AJ310480	atgttacgcagcagcaacgat	taagccgcgagagcgccaaca	281	(76)
aacC2,3	S68058	attcgaaaactcggagtc	cggagtggctccgaagtg	800	(152)
aadA1a	AJ009820	gtggtgatcgccgaagtatcgact	atagcgttaaggtttcattt	508	(152)
aadA2,3,8	AF261825	gtgaccatcgaaatttcgaac	atagcttcaaggtttccctc	406	(152)
aadA5,4	AF169041	ttttccctgcacaagttttca	gcaagattctctcaatcgttg	776	(152)
aadB	AF078527	gagcgaaatctgccgctctgg	ctgttacaacggactggccgc	321	(51)
abe_B	AE008792	tggtgcagttgggataatatcg	tctcttatctgttcgcctgttg	443	This study
acrF	NC_003197	cggctctgtcgtcaatctc	cgccagtaccataccaaacatc	436	This study
agfA	U43280	agcattcgcagcaatcgtag	ccaacctgacgcaccattac	402	This study
aphA1_IAB	AF024666	aaacgtcttgctcgaggc	caaaccgttattcattcgtga	461	(76)
armA	DQ177329	ggtcttactattctgcctatcc	ttctcttccattcccttctcc	511	This study
avrA	AF013573	cctgtattgttgagcgtctgg	agaagagcttcgttgaatgtcc	422	(152)
barA	AE008835	aataccgttgaccgcattcg	atgatcgccagcagattattcg	404	This study
bcfC	AE008694	caaccagagacattgccttcc	gtcgctgtcgctattctgatc	482	(152)
$bla_{oxa-1like}$	AJ238349	agcagcgccagtgcatca	attcgaccccaagtttcc	708	(89)
bla_{pse1}	AF153200	cgcttcccgttaacaagtac	ctggttcatttcagatagcg	419	(183)
$bla_{tem-1like}$	AF309824	ttgggtgcacgagtgggt	taattgttgccgggaagc	503	(90)
catA1	AY123253	tcagtcagttgctcaatgtacc	ggcgaagaagttgtccatattg	430	This study
cdtB	AL627271	caccagccgtcaggatataag	tcagaagcgagttgtggattac	477	This study
cmlA1like	M64556	tgtcatttacggcatactcg	atcaggcatcccattcccat	435	(91)
cmy-1(bla_{fox-1})	X77455	tgaggcgtaaggcgatgg	gcgggcataagtacaaggg	518	This study
cmy-2like	U77414	tatcgttaatcgcaccatcacc	gcctcttcgtaactcattcctg	489	(152)
copR	NC_003197	tgcctgtgatggacgagaac	cggacggtcataatgagcttc	549	This study
csgA	AE016840	agcattcgcagcaatcgtag	ccaacctgacgcaccattac	402	This study

cstA	AE008724	tattctgacggcggtggatg	agttgcgattcggtgtattgc	459	This study
ctx-M2	X92507	acagagcgagagcgataagc	cgttggtggtgccataatctc	488	This study
cutF	AY341107	tgtgcggattgtgaaggtattg	aaatgcccttcgaccgtcag	455	This study
dfrA1like	AY103456	gtgaaactatcactaatgg	ccctttgccagatttgg	473	(90)
dfrA12	AF175203	actcggaatcagtacgca	gtgtacggaattacagct	462	(91)
dfrA5-14	AJ313522	gatttctgcagtgtcaga	ctcaggcattatagggaa	379	(77)
dfrA17-7	AJ419170	cagaaaatggcgtaatcg	tcaccttcaacctcaacg	345	(77)
dgoA	AE008878	ggcggcagtacatgagtttg	caataaacagcggacgataggg	500	This study
entF	AE008723	ataatacggcggtaacgattcc	ggtatgatgcggtaacgacag	455	This study
envR	NC_003197	gcttacgggatgctggaatg	taattgcctcacactgccatc	403	This study
fhuA_Spa	Y14067	cgcactactggcatcacatc	gtcgtatccgtcgtgtattcg	413	This study
fhuA_STM	AE008703	cgtgattatccgtggcttctc	gttcaaccgttccctctttcg	497	This study
fimA	AE008721	gacctctactattgcgagtctg	agcgtattggtgccttcaac	450	This study
floR	AF118107	cacgttgagcctctatat	atgcagaagtagaacgcg	868	(91)
gipA	AF246666	acgactgagcaggctgag	ttggaaatggtgacggtagac	422	This study
glxK	AE008720	aattgattcgtcacgccctg	ttcaccgcctgcataacaatc	484	(152)
gogB	AE008818	ccttcctccgcatctatcttc	cacggctctattcttcctgtc	592	This study
gtgA	AF254762	ctatgatgaatgccgtgaggtg	ggcgattcttggtggtgatg	424	(152)
HCM1.71	AL513383	tcccgcagtcaatgaaataacc	ccagtggaaccagtgtcagg	443	This study
hilA	AE008831	aatacatcgtcgggagtttgc	ggtaaggcacgctcactattc	424	(152)
hilD	AE008902	acaaaaccgacgacaaaacatc	aaccacacatataagccactcg	421	This study
hin	AE008826	gcgcttactagtgcaaattgtg	gccgactaatctgttcctgttc	400	(152)
hdlD_DT104	AY462995	gtcaatgaccattgttctgttc	gttatgcacgatgattacactatc	162	This study
hsdM	AE008913	gacgacttcggcgatatgtac	gttggtggcgacgatatgtg	426	This study
htrE	AY144490	tgctgcttatcgctattctacc	atcttcgctattaccgcttgag	475	This study
hydH(stn)	L16014	agcgttcaggtacagattcaac	ttggcatcagcgttatcagc	445	(152)
iagB	AE008831	ccgagatggttcaaccgatc	gcccacaatgacagaaatgc	124	This study
int_SG1	AF261825	agatggagcgatattcagaagc	gcagtgaggacagcaagttc	474	This study
int1	X12870	gccttgctgttcttctac	gatgcctgcttgttctac	558	(91)
int2	L10818	cacggatatgcgacaaaaaggt	gtagcaaacgagtgacgaaatg	788	(155)
invA	M90846	gtgaaattatcgccacgttcgggcaa	tcatcgcaccgtcaaaggaacc	285	(194)
invH	AE008832	tttactgatcggctgtgctc	aggcttgcagtctttcatgg	407	This study

invI	AE008832	ggcgctgtacggtatttcattc	tcctctgactcggcctcttc	400	This study
iroB	AE008826	tgtcggtccaccaccactgtatg	gcggtaggcgttagaaagtg	439	(152)
irsA	AY328029	aatcggtgagtggatacacg	ttcagcacagccttatagacc	423	This study
IS150	X07037	agcgattaaggtcaagcgatac	aacactccaccacagcattatc	407	This study
ISCR1	AJ746361	atactaactggcgtgacaagag	agaatctgctcaatgaccttcg	166	This study
ISCR2	AF231986	cctcggatgcggtggatg	gccgttgtgctggtgttg	519	This study
ISCR3	AF261825	ggatgggctgcggatgag	ttgtggctttcgtgtgcttc	180	This study
ICSR4	AY341249	gccgtgcccgagtatgtg	cctcctcttcgagccaacc	583	This study
ISCR6	AY114142	atggatgggctgcggatg	cgatggcgtcaactgtgc	432	This study
ISCR7	AJ250371	tgtgtttccaccgctaccg	cagcagccgttccagtcc	520	This study
ISCR8	AF028594	gccatcgccttcattcacc	gcaccacgcttgtcactg	464	This study
leuO	AF106956	aacctattgaccgtgttcgatg	ttcctgatagcgtaactgatgc	408	This study
lpfD	AE008868	ccattgcgagcgatgtactg	tcattagcgtttggcattccc	496	(152)
marT	AF106566	cagccacagcatcagactatac	ttaaaccaacgccctgacatac	487	(152)
merA	K03089	accatcggcggcacctgcgt	accatcgtcaggtaggggaacaa	1232	(138)
mgtC	AF106566	tgactatcaatgctccagtgaat	atttactggccgctatgctgttg	654	(152)
misL	AE008875	gacgttgatagtctgccatccag	caatgccgccagtctccgtgc	989	(152)
mntH	AF161317	cctgatggcgatgctgattc	cggctaagaatacggcttcc	422	(152)
mrdA	AE008725	tatctacctgacgctggatctc	gtgaccatagccgaacttacc	499	(152)
msgA	AE008799	ccattaggttcctgctgtatcg	gtacggcttatctgttccttctc	205	(152)
nanH	NC_003197	aatgcttggcggtgttgg	aggtcctggaactcaatacttc	594	This study
oafA	AE008800	acgaagcacttagcaagaacg	caacagcaacaacaatgaggac	411	(152)
orgA	AE008831	gataaggcgaaatcgtcaaatg	gtaaggccagtagcaaaattg	550	(152)
oxyR	AE016845	ttgaatatctggtggcgttagc	tcatcaaacagcggcacttc	472	(152)
pagA(udg)	AE008792	tggtggcgttagacattgttc	acgatacgggagggataaagg	403	(152)
pagJ	AF013776	tcttacaatagcggcagactcc	ttcggggaagggcacgtc	106	This study
pagK	AE008783	accatcttcactatatcctgctc	acctctacacattttaaaccaatc	151	(152)
parA-parB	AF250878	ggagcgatggattacttcagtac	tgccgtttcacctcgtgagta	471	(36)
pefA	AB041905	cctgtgacctgaccacttctg	ttgtaagccactgcgaaagatg	418	(152)
pflD	AE008892	ttctctgcctggatgcctac	ttgctgccttctaccattagtg	406	(152)
phoP	AE008753	attattacgccaccacctgaag	atctttgctgaccactttaccg	487	(152)
phoQ	AE008753	atgcaaagcccgaccatgacg	gtatcgaccaccacgatggtt	301	(89)

pilR	NC_004631	ggtatgttcagtggtcggttac	agaagcaaagcggtggataac	474	This study
pilV	NC_004631	tcactcaggcagtctcatcc	caggtcatccgtgctcaac	410	This study
pipA	AE008747	tgacgacacttatgacgaatgc	ttctgggaggtgaaggatgc	424	(152)
pipB2	AY532917	tgacagaggtgctccgttg	cttccagacaggcgttcttg	437	This study
pipD	NC_003197	tggtaggcaatcaggcttcg	ctggcaggatcacggactc	357	This study
prgH	NC_003197	acggctgtgagtttccattg	ctgcggcgagttaagtatcc	428	This study
prot6E	U66901	gcctaaggttagtgtgactctc	ctagcagccgttggtatcc	579	(152)
qacEΔ1	AF261825	atcgcaatagttggcgaagt	caagcttttgcccatgaagc	250	(209)
qnrA	AY906856	cgccaggatttgagcgacag	tctgagcccatcaaggaagc	431	(152)
qnrB2_B1	AM234698	tggctctggcactcgttg	agatcgcaatgtgtgaagtttg	526	This study
qnrS	AM234722	caacaatgccaacttgcgatg	ccagtgcttcgagaatcagttc	427	This study
ratB	NC_003197	acaatacgactcttcacgcatc	attctcatctacgccgcaatac	410	This study
rck	AE006471	ctgtcctcactgctgctgtc	cttccctcatagcccagatcg	485	(152)
recC	AE008837	aggatgttctggcgttgttag	ccactgctgttcaatcaatgtc	446	(152)
rep_iterons_FIA	J01724	ccatgctggttctagagaaggtg	gtatatccttactggcttccgcag	462	(36)
rep_iterons_HI2	BX664015	tttctcctgagtcacctgttaacac	ggctcactaccgttgtcatcct	644	(36)
rep_iterons_P	M20134	ctatggccctgcaaacgcgccagaaa	tcacgcgccagggcgcagcc	534	(36)
rep_oriγIncX	Y00768	aaccttagaggctatttaagttgctgat	tgagagtcaatttttatctcatgttttagc	376	(36)
rep_RNAIInc1	M20413	cgaaagccggacggcagaa	tcgtcgttccgccaagttcgt	139	(36)
rep_RNAIB0	M28718	gcggtccggaaagccagaaaac	tctgcgttccgccaagttcga	159	(36)
rep_RNAIK/B	M93063	gcggtccggaaagccagaaaac	tctttcacgagcccgccaaa	160	(36)
rep_SG1	AF261825	caccctcgcccaatgatttc	accttgattgtcgctgttacc	458	(152)
rep_W	U12441	ggtgcgcggcatagaaccgt	cctaagaacaacaaagcccccg	242	(36)
repA_A/C	X73674	gagaaccaaagacaaagacctgga	acgacaaacctgaattgcctcctt	465	(36)
repA_B_CIncL/M	U27345	ggatgaaaactatcagcatctgaag	tgagagtcaatttttatctcatgttttagc	785	(36)
repA_FIB	M26308	ggagttctgacacacgattttctg	ctcccgtcgcttcagggcatt	702	(36)
repA_FIIs	AE006471	ctgtcgtaagctgatggc	ctctgccacaaacttcagc	270	(36)
repA_N	NC_003292	gtctaacgagcttaccgaag	gtttcaactctgccaagttc	559	(36)
repA_repBIncF	AY234375	tgatcgtttaaggaattttg	gaagatcagtcacaccatcc	270	(36)
repA_T	K00053	cgttgattacacttagctttggac	ttggcctgtttgtgcctaaaccat	750	(36)
repA_Y	K02380	aattcaaacaacactgtgcagcctg	gcgagaatggacgattacaaaacttt	765	(36)
repA2_FIC	M16168	gtgaactggcagatgaggaagg	ttctcctcgtcgccaaactagat	262	(36)

repC_DT193	AY524415	ctaagaacaagcacagcctcag	tgggcgatgatggataccttc	439	(152)
repC_R64	AP005147	tacttcggtcaacgccctag	gaggtgttcggcatacatctg	427	(152)
rfbD_A/B/C2-C3/D1/D2	AE008792	tggtaagacagggcaagtagg	caactggtgcggaagataagg	458	(152)
rfbE_A/D	AL627273	agtggtgttcaggcattcatc	gctccagtcaattgcattagtg	400	(152)
rhaA	AE008889	gtcggctccaatgagttctac	acgctggcaatacatttccc	468	(152)
rhuM	AE008874	acaagttcaacaggaaggtagc	aatgagttcagcagcggtatg	409	(152)
rpoS	AE008833	gattgagagtaacctgcgtctg	catcttgcgtggtgtcttcc	476	(152)
safC	AE008708	gaagtggcaggcagcatatac	cacggcagaccatacatcaac	495	(152)
sat(Tn7)	M63169	tatccgaccaaggctttgaac	cgagaaccagtaccagtacatc	416	(152)
sat1(int2)	AB161461	gccaggtcaagctccatattc	tctctcgccaccacctaaag	433	(152)
SB10	NC_004313	gcgttcgtcaaaattggtcag	ttcgctgaggacggagtg	421	This study
SB54	NC_004313	tgtcaatggtgtgtccgttc	ctgattgccagtagttcaacac	258	This study
sefA	L11008	ttcaggcagcggttactattg	ttgatactgctgaacgtagaagg	401	(152)
sefR	AF239978	aggcttctaattgctcagaaag	atacgcttgacattcctacgg	459	(152)
SEN4287	xxxxxx	aatccacatccagcagttcc	atcaccaccattaccgaagtag	277	(152)
sfbA	AE008719	atgggattgcgtcagagtttac	aggttagtcggatcgttggag	422	(152)
sgbE	NC_003197	tgaccgccgacgatatgg	gaccgcgctcttcaaagg	321	This study
shdA	NC_003197	gatgacacgccttcggatg	gccaacggtaacgctctg	402	This study
sifA	U51867	gccaccattattcttcgcatc	tttaatccacacatcgccattg	455	(152)
sipA	AE008831	aggctgtgaccaacgcttc	ggaacggtgtggaggtatctg	480	This study
sirA	AF026035	aaagaatttcgtctcgcccac	tcggcattgttactggcattc	82	(152)
slrP	AF127079	gtgaaggacctcaacctacaag	cagttccgctaagtggctatc	500	(152)
slyA	AE008762	gccaaaactgaagctacaggtg	cggcaggtcagcgtgtcgtg	701	(89)
sodC1	AF007380	gttcagcaatggcagagaatac	ccagtggagcaggtttatcg	444	This study
sodCIII	AF254764	cagcctcagcagcaagtac	gcgggttgggatgatcttc	481	This study
sopA	AF121227	ccgtgaagttgattgatgatgc	caggatggatgacagaacactc	428	(152)
sopB	AE008747	gatgtgattaatgaagaaatgcc	gcaaaccataaaaactacactcac	1169	(152)
sopD	AE008834	tgttacactccatcattcacgg	gtaagtgagtcctgccattcg	460	(152)
sopD2	AL627268	ggctaacccgtttgatgagtc	accattccattcaacacattcc	559	This study
sopE1	AE016848	cgggcagtgttgacaaataaag	tgttggaattgctgtggagtc	455	(152)
sopE2	AF200952	tgagggtagggcggtattaac	ctgaaattgttgtggcgttgg	460	(152)
spaS	AE008832	tgcctatctggtgtcgtatgg	ccaatgcgaggagaagttcac	431	(152)

siiD(spi4_D)	AJ576316	gaatagaagacaaagcgatcatc	gctttgtccacgcctttcatc	1132	(152)
siiE(spi4_F)	AJ576316	cggcggaagagaatgctaag	gcggcgtagtaagcgtatag	402	(152)
siiF(spi4_R)	AJ576316	gcaatggcgatatctctatccg	ataataactgctgctgctgtcc	450	(152)
sprB	NC_003197	ggtagtggcagatcgcttg	gtcaggtacagcagggttatg	487	This study
sptP	AE008831	aggagatggcttcaggtaacg	tattcactggcacaggcattc	408	(152)
spvC	AE006471	gctggatgtgcctgactattc	cgcccacttcgtagttcattac	453	(152)
spvR	AE006471	catttcagcactgaccatttcc	ataatcccagagcccgacag	428	(152)
srfJ	AE008907	ttacagcagattgacggattcg	cggtattccagcaggtagttg	422	(152)
ssaQ	AE008761	gaatagcgaatgaagagcgtcc	catcgtgttatcctctgtcagc	643	(152)
sseC	NC_003197	tggcgaggaagtggttgag	gcacatctgatagcctgtaagc	402	This study
sseF	AE008761	gtgctgcttacgcctgaac	gccatttgttccgccaataac	581	This study
sseI	AE008743	tcccgccatcatcagtaacc	tctcctgactgtcttcatccag	290	(152)
sseJ	AF294582	acagacaagtcgcatcttacac	catagccgtggtgagtataagg	408	(152)
sseK1	AE008894	ggaatcctgctcgctttgg	tcataacacccaaccttatccg	475	This study
sseK2	AE008795	acgctcagtccaccttcac	gcaagacctcatctcgttcac	412	This study
sspH1	AF013776	tgccgtcaggactacagaag	cagttgtgtcagccaggatg	470	This study
sspH2	AE008800	actgtcgctgtcggtctatc	ttgctcttaacgcctcatcttc	431	(152)
ssrB	AE008761	tcctcaattacatcagcgttgg	tcattcttcgggcacagttaag	419	(152)
staA	AL627265	gcgactgatggtgacgtatatc	tgcgttcgtggttacatgac	414	(152)
stbD	AE008710	tttgccaacggtgattagcg	tccaggtatctcggtccattg	461	(152)
stcC	AE008795	aattacggcgtgagtgttagtc	aagaggcgaaccatcaggac	480	(152)
stdB	AE008839	cgtatctgttccgtgccttg	tgaccgtgctgttgttatgc	438	(152)
steB	AL627276	gtgattgcggatgtcggaag	ttgcgtatgttcctggattagc	474	(152)
stfE	AE008703	gggaagtaagacggtcaacatg	ggtaatccaccaccaacgtg	431	(152)
stgA	AL627280	ccttggtgaagtggttgacag	tgaactgagaatctgcgttagc	467	(152)
stiC	AE008702	aataccagtccgtcgccttc	cgtcgtcgttgtcatcatcg	489	(152)
stjB	AE008915	gtgataccaccagcgactattc	ggaagccattatactgcgagag	458	(152)
stkC	NC_006511	aatgcgttgaaatctacctctg	tgcggtcatcgttatacatcc	592	This study
STM0305	AE0098709	cggaaacaggacggggctgt	ccgaaggcgcaatggaggat	155	This study
STM0330	AE008710	ccgatgctggaaccgaatatc	tttcctctgccgtcgtaatttc	430	This work
STM0900	AE008737	ctgattaagccgaccaccatc	ttgcgtagatgatacagcgaac	439	(152)
STM1896	AE008784	cagcggcggaactcataaac	ttaggcaaacgagcaaccatg	435	(152)

STM2616	AE008819	ctttccccgattacccataacc	tgactatccagccgctatcg	496	(152)
STM2701	AE008823	atattatcggcggcgtaacttc	tgcgaatcaatgtggtgacg	492	(152)
STM2740	AE008824	ccgaagatgctcaaggaacac	ggctccatgatctgaagaacg	437	(152)
STM3098	AE008842	tttggcggcgcaggcgattc	gcctccgcctcatcaatccg	423	This study
STM3782	AE008876	acctctgtctgaagccattaag	ggttaccgccatcaaggaag	404	(152)
STM4057	AE008889	ggtggcctcgatgattcccg	cagcgtaccgttggaataaatc	211	This study
STM4200	AE008896	taatcacacgcatacctggaac	ttgtcatacgccgcctttac	483	(152)
STM4210	AE008896	agagatgatggatgaggtggc	gtaatatcggcagcggcaag	426	(152)
STM4497	AE008911	tcgatcccgtgcttgaatac	cgtgtccgctataggttcc	501	(152)
STM4595	AE008916	tttgcccagaacacgattacg	gcggcgtaataggtaaagtctg	407	(152)
strA	M28829	atcgtcaagggattgaaacc	ggatcgtagaacatattggc	548	(147)
strB	M28829	cctggtgataacggcaattc	ccaatcgcagatagaagg	509	(147)
STY3672	AL627279	aagtgtgctgctgagttatgtc	tagcggcatgatcgtatgttg	430	(152)
STY3676	AL627279	gtgaatggctggaacagatgg	ctgcggcggtaaagagattg	417	This study
STY4221	AL627281	cgcctttactgatgacgatgg	tcaccacctcttcacgatagtc	600	This study
STY4625	AL627283	atgcgagcagaccaactttg	ccagcgggaaatacttgtcag	419	(152)
STY4631	AL627283	gcagaagcacacgcatcc	ttcttcctttagcgggtttgg	314	This study
sugR	AF106566	ggtaatggtctggcgtcaatac	gtagcggatggtgaacatatcg	414	(152)
sul1	X12869	cttcgatgagagccggcggc	gcaaggcggaaacccgcgcc	436	(209)
sul2	M36657	tcaacataacctcggacagt	gatgaagtcagctccacct	707	(41)
sul3	AJ459418	gagcaagatttttggaatcg	catctgcagctaacctagggctttgga	789	(183)
tcfA	AL627266	cacctcgctgttcgtctattc	gccgtgtgtttcccgtattc	401	(152)
tet(A)	X61367	gctacatcctgcttgcct	catagatcgccgtgaaga	210	(91)
tet(B)	J01830	ttggttaggggcaagttttg	gtaatgggccaataacaccg	659	(175)
tet(C)	J01749	cttgagagccttcaacccag	atggtcgtcatctacctg	210	(91)
tet(D)	L06798	aaaccattacggcattctgc	gaccggatacaccatccatc	600	(175)
tet(E)	L06940	aaaccacatcctccatacgc	aaataggccacaaccgtcag	278	(174)
tet(G)	S52437	gctcggtggtatctctgc	agcaacagaatcgggaac	468	(174)
tnp_cf	NC_006816	gaccagtgcttggatctcag	atcaggtaattcgtggagtagg	578	This study
tnp_IS1	NC_006816	tcacttacaccgcttctcaac	cagataatgcccgatgactttg	551	This study
tnp_IS102	NC_003384	gtgtcagtaagcgggcaaag	cgagcgacgggtgtaatctg	485	This study
tnp_IS1202	NC_006816	gctatgtgcggagaccag	tctgcgggaaggctcatc	559	This study

tnp_IS1294	NC_003198	cggagtcgttgtcacatatcac	gccacacagcacgcattc	473	This study
tnp_IS1351like	NC_002305	ggctccaccgtatttactgtc	ccgtaatttcccgcacctc	460	This study
tnp_IS30	AJ310778	gacggtcgcatagccttc	agtgtctttctcggtctgttg	529	This study
tnp_Orf341	AJ634602	acacctgcctcgctacatc	ttgcttgccatagtcatcttcg	475	This study
tnp_pFPTB1	NC_006511	acgccagtcagaacaatcaatg	gcaatgaacggaccagcac	457	This study
tnp_Spa2465	NC_003198	ctggcactggctgaatgg	tcctccggtatcatattgttcc	233	This study
tnp_STY343	NC_003384	gcaccagatcatcgctgtg	ccgccgtatccagacttcc	117	This study
tnp_Tn2680	NC_003384	atcgcattcaatcacgttgttc	gatggagctgcacatgaacc	538	This study
tnpA_IS1like	NC_006816	gtggcttctgtttctatcagc	tttacggcagtgagagcag	111	This study
tnpA_IS1696	AJ746361	ccgagttgctgatggaggtc	ggtgaagcctgccgtgtc	539	This study
tnpA_IS186	AY341107	ccgactggtttggcatacttg	caccgttcttaccgcaatcc	420	This study
tnpA_IS200	NC_006816	ggggacgaaaagagcttagc	taaacaggctgcccggatac	442	This study
tnpA_IS26	AY509004	cgatcactccacgatttaccg	gcacgcatcacctcaatacc	495	This study
tnpA_IS3/IS911like	AY509004	cggtatggaggtgcctgaag	cagtactttcgcccaagagc	129	This study
tnpA_IS4	AY509004	aacgatagaatctcttggatgc	cgaaaggtctttatttgctgtc	489	This study
tnpA_IS406	AF261825	cgccgaaatgtatgtcaaagg	tccagggaagacagcatcg	418	This study
tnpA_IS6100	AF071413	cgctggtattgtcgctatcc	cggcctcgatcacgttattg	466	This study
tnpA_Tn21	AY509004	cattcaacgataccgacctctc	cgcaaccaagccaaccag	547	This study
tnpA_Tn3	AP005147	gacctcggcgaaaccaag	gttccacagcacgatagcc	447	This study
tnpR_IS10	AF261825	agcggaaaactggaaacctatc	ctgactgtgttagcctggaag	483	This study
tnpR_SG1	AY144490	tttcaaccgacgatcagaacc	cgcctagcaacatcagatacag	484	This study
tpase1	AE006471	gttacgatttccctgaagaagc	aaatggggtattagggtgtgtc	281	This study
traT	AE006471	agtgaacggacggtgtatctg	cacacgggtctggtatttatgc	495	This study
trhH	AF261825	aactggtgccgttgtcattg	gatggtctgtgcttgctgag	418	This study
ttrC	AF282268	gtgggcggtacaatatttctttt	tcacgaataataatcagtagcgc	921	(153)
wbaA_C1	M84642	gttggcagactggtactgattg	atagaatggcaccaagcacaag	500	(91)
wbaO_E1/D2	X60665	ggcagtcaagtcgatgttattg	gtactccaagatcagcaacctc	493	(152)
wbaU_B/D	X56793	tagctgttggtgcaagttgg	tcataccgtgcgacatagttg	448	(152)
wbaV_B	X56793	gagaccgggtgtgatttagttg	ttgccataacatgccatagcc	488	(152)
wbaV_D1	M65054	tcggcgatggttaaatggtg	gcaagttcagctctatcggtag	474	(152)
wcdA	D14156	aagaagaatgcgtgccgatc	atacagtgaccaccgaccag	464	(152)
wcdE	D14156	agtcatctgcctcaatcattcg	aaatcaatcgctcgccgtag	424	(152)

wzf	D14156	aagagcgaaggtagcacaag	ccatccgatccatcatttctttc	428	(152)
wzxO6,14	AY334017	cgttcattaggagagcaggttg	atcccaagcatcaaacttcgc	414	(152)
wzy(O27)	AF017148	ggcaagtcgtgtctggaatatc	tgtagccaatccagcatccg	393	(152)
wzy_B/D	AE008758	ccagtgttgatggagttcagag	gccaaatagccctcggaatc	496	(152)
wzy_D2/E1	U04165	ccgctgcatatggtggttac	tgcgttctcacttcattagcc	478	(152)
yafD	AE008706	ttgccaccgctaactatcttg	cagtacggaggcttcattcac	476	(152)

Table A2: Table of DNA microarray probe sequences for the detection of the presence of genes in *Salmonella enterica* subspecies *enterica* serovars designed by this study

Accession No.	Probe name	Marker group	Gene function	Probe sequence	Length
X01385	aac(3)-IV	Resistance	Aminoglycoside-3"-acetyltransferase, encoding GEN resistance	GACACGATGCCAACACGACGCTGCATCTTGCCGAGTTGATGGCAAAGGTTCCCTATG	57
AJ310480	aacC1	Resistance	Aminoglycoside-3"-acetyltransferase, encoding GEN resistance	CTTATGTGATCTACGTGCAAGCAGATTACGGTGACGATCCCGCAGTGGCTCTCTATA	57
S68058	aacC2,3	Resistance	Aminoglycoside-3"-acetyltransferase, encoding GEN resistance	GAAGAAACGGTGAAGTCGCCTGGAAAACGGCATCAGAATACGATTCAAACGGCATTC	57
AJ009820	aadA1a	Resistance	Aminoglycoside-3-adenyltransferase, encoding STR/SPE resistance	GAAGTGGTGATCGCCGAAGTATCGACTCAACTATCAGAGGTAGTTGGCGTCATCGAG	57
AF261825	aadA2,3,8	Resistance	Aminoglycoside-3-adenyltransferase, encoding STR/SPE resistance	AAATTTCGAACCAACTATCAGAGGTGCTAAGCGTCATTGAGCGCCATCTGGAATCAA	57
AF169041	aadA5,4	Resistance	Aminoglycoside-3-adenyltransferase, encoding STR/SPE resistance	GTTCTTGCTCTTGCTCGCATTTGGTACAGCGCTTCAACTGGTCTCATTGCTCCTAAG	57
AF078527	aadB	Resistance	Aminoglycoside-2"-adenyltransferase, encoding GEN resistance	CATGGAGGAGTTGGACTATGGATTCTTAGCGGAGATCGGGGATGAGTTACTTGACTG	57
AE008792	abe_B	Serotyping	CDP-abequose synthase: Serogroup B	ACCTTCATATACTGAGTATCAAGTTGGAACTGGTGCTGGGGTAAGTTTGAAAGATTTTCTGGT	63
X61917	abe_C2-C3	Serotyping	CDP-abequose synthase: Serogroup C_2-C_3	TGCATTAAAGCGTCCTATAACCGAGCCAACGATTATCAATACCTTGATTGAATGGTTGA	59
NC_003197	acrF	Resistance	Acridine resistance protein F	GACATCTCTGACTATGTCGCCTCTAACATTAAGGATTCTATCAGCCGTCTGAATGGT	57
U43280	agfA	Fimbrial	Thin aggregative fimbriae precursor	GAACTGACTCAGAATGGTTTCAGAAATAATGCCACCATCGACCAGTGGAACGCTAAA	57
AF024666	aphA1-Iab	Resistance	Aminoglycoside-3"-phosphotransferase, encoding KAN resistance	TTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGAC	57
DQ177329	armA	Resistance	16S rRNA Methylase, extended-spectrum beta-lactamase-resistant	AAAGTCTTTATCTGGAAAGGAGAAGGGAATGGAAGAGAATTACCAGCTATGGTTTGAATCTTT	63
AF013573	avrA	Pathogenicity	SP1 encoded protein, inhibits the key proinflammatory	ACCGAAGCATTGACCTGTATTGTTGAGCGTCTGGAAAGTGAAATTATAGATGGCAGC	57
AE008835	barA	Pathogenicity	Sensory histidine kinase	GCGGCTCGACCTTCTGGTTTCATATTAATCTTGATCTTAACCCAAATGTCATTATTGACGGGC	63
AE008694	bcfC	Fimbrial	Fimbrial usher, bovine colonization factor	GCACAGTCAGGAACCAATTTACAGCTTATGGGCTATCGCTATTCAACCTCGGGCTTT	57
AJ238349	$bla_{oxa1\ like}$	Resistance	Extended spectrum ß-lactamase, encoding AMP resistance	TTCTCTGGAGATAAAGAAAGAAACAACGGATTAACAGAAGCATGGCTCGAAAGTAGCT	58
AJ238349	$bla_{oxa1\ like}$	Resistance	Extended spectrum ß-lactamase, encoding AMP resistance	CCCAAAGGAATGGAGATCTGGAACAGCAATCATACACCAAAGACGTGGATGCAATTT	57
AF153200	bla_{pse1}	Resistance	Extended spectrum ß-lactamase, encoding AMP resistance	AGTATTACAGCAGTTGTGTGGAGTGAGCATCAAGCCCCAATTATTGTGAGCATCTATCT	59
AF153200	bla_{pse1}	Resistance	Extended spectrum ß-lactamase, encoding AMP resistance	GCAAGTTGAACAAGACGTTAAGGCAATTGAAGTTTCTCTTTCTGCTCGTATAGGTGTTTCCG	62
AF309824	$bla_{tem-1\ like}$	Resistance	Extended spectrum ß-lactamase, encoding AMP resistance	TAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGG	57
AF309824	$bla_{tem-1\ like}$	Resistance	Extended spectrum ß-lactamase, encoding AMP resistance	AGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATC	57
AY123253	catA1	Resistance	Chloramphenicol acetyl transferase, encoding CHL resistance	ACATATATTCGCAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTATTTCCCTAAAG	57
AL627271	cdtB	Pathogenicity	Cytolethal distending toxin, secreted protein	GGAATCTTCAGGGCTCTTCAGCATCTACAGAAAGTAAATGGAATGTCAATGTCAGAC	57
M64556	cmlA1like	Resistance	Chloramphenicol exporter, encoding CHL resistance	AAATATGGGCTTTGCAGTCCGTGTTAGGCTTTATTGCTCCAATGTGGCTAGTGGGTATT	59
X77455	cmy-1 (bla fox-1)	Resistance	CMY-type extended spectrum ß-lactamase, encoding AMP resistance	GAGTTCAGAAGAGCTAAGAAGTTGCTTGAGGTACTGGGTTGCATTGATAATAGTCATG	58
U77414	cmy-2 like	Resistance	CMY-type extended spectrum ß-lactamase: AMP resistance	CTATTCCGGGTATGGCCGTTGCCGTTATCTACCAGGGAAAACCCTATTATTTCACCT	57
NC_003197	copR	Resistance	SPI-5, Copper resitance protein	CAGGAACATTATTCATTGATTATTCTTGATATTATGCTGCCGGGGCTTGATGGATGG	57
AE016840	csgA	Fimbrial	Major curlin subunit precursor	TAGGCCAGGGTGCGGATAACAGTACTATTGAACTGACTCAGAATGGTTTCAGAAACAATG	60
AE008724	cstA	Metabolism	Carbon starvation protein	ATGAATAAATCAGGGAAATACCTCGTCTGGACAGCGCTCTCAGTATTGGGTGCGTTT	57
X92507	ctx-M2	Resistance	CTX-M2 extended spectrum ß-lactamase: AMP resistance	TATAGCGACAATACTGCCATGAATAAGCTGATTGCCCATCTGGGTGGTCCCGATAAA	57
AY341107	cutF	Metabolism	Putative copper homeostasis protein	CAGCCTGTACGCTATTTGCATTGATAGGGTGTAATAACCGTGCGGAAGTTGACGCCC	57
AY103456	dfrA1 like	Resistance	Dihydrofolate reductase, encoding TMP resistance	ACCCAACCGAAAGTATGCGGTCGTAACACGTTCAAGTTTTACATCTGACAATGAGAAC	58

AF175203	dfrA12	Resistance	Dihydrofolate reductase, encoding TMP resistance	CGGCAAGCCTCTACCGAACCGTCACACATTGGTAATCTCACGCCAAGCTAACTACCG	57
AJ313522	dfrA14	Resistance	Dihydrofolate reductase, encoding TMP resistance	GGTCGTTACCCGCTCAGGTTGGACATCAAATGATGACAATGTAGTTGTATTTCAGTC	57
AF220757	dfrA17,7	Resistance	Dihydrofolate reductase, encoding TMP resistance	TCTTCCAAATCGCAAATATGCAGTAGTGTCAAAGAACGGAATTTCAAGCTCAAATGAA	58
AE008878	dgoA	Metabolism	Galactonate dehydratase	GAAAATAACTCACATCACCACGTACCGTTTACCTCCACGTTGGATGTTCCTGAAAATCG	59
AE008723	entF	Pathogenicity	Enterobactin synthetase, component F (nonribosomal peptide synthetase)	CGCTATTTGGCCCGGTGCTCAACATAAAAGTGTTTGATTATCATCTGGATCTTCCTGG	58
NC_003197	envR	Pathogenicity	Transcriptional repressor, envelope Regulator for envCD, acrEF	ACTTATTCAGGATAGGCTTACGGGATGCTGGAATGATAATCCTTTACAGGATCTACG	57
Y14067	fhuA_Spa	Pathogenicity	Outer membrane protein receptor	GTTACGACTGGGCCGATCAAGAGTCTCTTAACCGCACTACTGGCATCACATCTAAAC	57
AE008703	fhuA_STM	Pathogenicity	Outer membrane protein receptor	GTTGACAACGAGCGTTTACAGAATTTCAGCGTAGATACACAACTGGAAAGTAAATTCGC	59
AE008721	fimA	Fimbrial	Major type 1 subunit fimbrin (pilin)	GACAATAGCACTACCGCAACCGGCGTCGGGATTGAGATTCTTGATAATACCTCTTCA	57
AE008787	fliC/fljB	Serotyping	Filament structural protein, detects all H antigens	CACAAGTCATTAATACAAACAGCCTGTCGCTGTTGACCCAGAATAACCTGAACAAATCCC	60
AY649698	fliC_b	Serotyping	Filament structural protein, detects b antigen	ACAGTTACTGAAAACCAAATTGTAGACGCTGTTACACCGACGCCAGTTGATACAGTC	57
AL627272	fliC_d	Serotyping	Filament structural protein, detects d antigen	AACCAAATTGCTGAAGTAACAAAAGAGGGTGTTGATACGACCACAGTTGCGGCTCAA	57
AJ292284	fliC_e,h	Serotyping	Filament structural protein, detects e,h antigen	CTTGAAGCCGGTGGCAAGTACTATGCTGCAACCTATGACGAAGGTACAGGTAAAATC	57
AJ292278	fliC_e,n,x-e,n,z15	Serotyping	Filament structural protein, detects e,n,x and e,n,z_{15} antigens	CCCAACTAAATCTACTGTTACAGGTGATACCGCTGTTACTAAGGTACAGGTTAATGCTCCT	61
M84980	fliC_g,x	Serotyping	Filament structural protein, detects g complex associated antigens	GTACCGCTGAAGCCAAAGCGATAGCTGGTGCCATTAAAGGTGGTAAGGAAGGAGATA	57
AE008787	fliC_i	Serotyping	Filament structural protein, detects i antigen	GGTCTTGGTGGTACTGACCAGAAAATTGATGGCGATTTAAAATTTGATGATACGACTGGA	60
U06201	flic_m,t	Serotyping	Filament structural protein, detects m,t antigen	CAACTCAGGGGCGGTAGTAACTGACACCACTGCTCCAACTGTTCCTGATAAAGTATA	57
X04505	fliC_r	Serotyping	Filament structural protein, detects r antigen	AAGTCACTTTAACTGGCACACCAACAGGACCAATTACTGCTGGCTTCCCTTCAACTG	57
AY434692	fliC_z10	Serotyping	Filament structural protein, detects z10 antigen	AAACTGCTGGAATTACTGGTGCTACATTAAAAGCTGGTATTACTGGTACAACGACAGAAACCG	63
AY649736	fliC_z4,z23	Serotyping	Filament structural protein, detects z4,z23 antigen	AAATTAGATGTGACTAAAGGAATCGCAACCACTGTAAGCTCTGGAGCCTCGGTAGTT	57
AE008826	fljA	Serotyping	Repressor of phase-1 flagellin	GTGTGAGGACATCCAATGGCAATCATATGCAAGTTATGTTTGACTGGGTGAGCAGGA	57
AE008826	fljB_1,x	Serotyping	Filament structural protein, detects 1,x antigens	CCAATAATGGTACTACACTGGATGTATCGGGTCTTGATGATGCAGCTATTAAAGCGGCT	59
AJ292277	fljB_l,w-l,v	Serotyping	Filament structural protein, detects l,w and l,v antigens	ACAGTGGTATTAGTGCTGCTGATGCTGCAAAAGGTCAATTAGTTACGATGTCTTATACGGA	61
AF118107	floR	Resistance	Putative efflux protein: FLO/CHL resistance	CTGATGGCTCCTTTCGACATCCTCGCTTCACTGGCGATGGATATTTATCTCCCTGTC	57
AF246666	gipA	Pathogenicity	Gifsy-1 Peyer´s patch-spezific virulence factor GipA	CTGATTAACGATAACCAAGTTGTATGCGTCGAATCCCTGAAAGTGAGGAACATGATC	57
AE008720	glxK	Metabolism	Glycerate kinase II	GAAACGGGTTTCTGTGGACGTTAGCGGGCCGATGGGGGAAAAAGTAAACGGATTTTAT	58
AE008818	gogB	Pathogenicity	Gifsy-1, gifsy one gene, leucine-rich repeat protein	TGGGACAGGAAGAATAGAGCCGTGTTTAATAAAGATGAGAAGATAGCAGAAAGATTGAATGA	62
AF254762	gtgA	Pathogenicity	Gifsy-2 prophage protein	TTCCAGACCTTCCAGAACACCAAGATAATCCTTCGCAATTACGCCTCCAACATGATG	57
AL513383	HCM1.71	DNA-Mobility	Putative periplasmic protein, on pHCM1 plasmid (R27)	CCATGTAATTTCAATATGTACGCCAAGTTAGGTCAAAGACTGGTGGGATCTCGGGAA	57
AE008831	hilA	Pathogenicity	SPI-1 encoded transcription activator	TGATGATTTCATACTCAACATGGACGGCTCCCTGCTACGCTCAGAAAAGAAAGTCAATA	59
AE008902	hilD	Pathogenicity	SPI-1 putative AraC-type DNA binding domain containing protein	ACTTTTCGGCCCCATTAACAAAACCGACGACAAAACATCTGTTAGCGCCAATAGAAA	57
AE008826	hin	Serotyping	Regulator for fljA: DNA invertase hin	TACATGAACGTGGAGCTCACTTCCATTCTTTAACCGATAGTATTGATACCAGTAGCGCG	59
AY462995	hldD_DT104	Pathogenicity	DT104 specific phage encoded protein	AAAGGTCAATGACCATTGTTCTGTTCATCGCATAGGTTTCAGCAGACTCTATAAGCG	57
AE008913	hsdM	Metabolism	DNA methylase M, host modification	AAAACTACGTCAATGAACTCGCCTCGCTGCTGTTTTTGAAAATGTGCAAAGAGACCG	57
AY144490	htrE	Pathogenicity	Probable porin/fimbrial assembly protein	TTGTGCGTGGTATTAACAATGCTGGTGAACTCATCGTTCGTTGGTATGAAGAAGGTC	57
L16014	hydH(stn)	Pathogenicity	Enterotoxin sensory kinase	ATTCAGGGAGTGAGTAATAATATCATTGAGGTTAACCGTCTGGAGCGTCAGATGCGC	57
AE008831	iagB	Pathogenicity	SPI-1, invasion associated protein IAGB precursor	CATAACCGAGATGGTTCAACCGATCTTGGCCTGATGCAAATTAACAGCTTCCATATG	57
AF261825	int_SG1	Resistance	Integrase from Tn4555, present in *S.* Typhimurium DT104 SGI 1	CTATCTCTACGAGAACCCAAAGACACAAGCAGAGCGTCAGCACAATAAAGAAATGTTGC	59
X12870	int1	Resistance	DNA Integrase1: Integron associated	GATCTGCTCGGCCATTCCGACGTCTCTACGACGATGATTTACACGCATGTGCTGAAAG	58
L10818	int2	Resistance	DNA Integrase2: Integron associated	GCAAGAACTCTTAGGGCATAACGATGTTAAGACCACGCAAATCTATACGCATGTGTTGG	59

M90846	invA	Pathogenicity	SPI-1 encoded invasion protein	TGTTCGTCATTCCATTACCTACCTATCTGGTTGATTTCCTGATCGCACTGAATATCGTACTGG	63
AE008832	invH	Pathogenicity	SPI-1 encoded invasion protein	AACCCGGAAGTAAGAATTTAAGCATATATCAGACGTTACTTGCTGCCCATGAAAGACTGCAA	62
AE008832	invI	Pathogenicity	SPI-1 encoded secretory protein	CAGACAGCTCAGTCGTGAGGAAATTTATACGTTATTACGTAAGCAGTCTATTGTTCGCCGG	61
AE008826	iroB	Pathogenicity	Putative glycosyl transferase	GTCGTTGGACCACTGATTGCCGCTAAGTATGACATTCCGGTAGTGATGCAAACCGTC	57
AY328029	irsA	Pathogenicity	Putative transcriptional regulator, internal response element to stress	GGTCTATAAGGCTGTGCTGAACGTCCTGTGGAACTGGATTCTGTTTCGTAAATTTTC	57
X07037	IS150	DNA-Mobility	Insertion element, unnamed protein ORF B	TTGTCTGGATAATGCTGTGGTGGAGTGTTTCTTTGGAACCTTAAAGTCGGAGTGTTT	57
AJ746361	ISCR1	DNA-Mobility	Insertion sequence common region ISCR1	GAAGATCCGAAGGTCATTGAGCAGATTCTCAAGCATCTGAAACAGAAAACAGCCAAG	58
AF231986	ISCR2	DNA-Mobility	Insertion sequence common region ISCR2	GATCGGCGCTCAATCTGAATGTTCACTTCCACATGCTGTTTCTCGACGGTGTGTATG	57
AF261825	ISCR3	DNA-Mobility	Insertion sequence common region ISCR3	CGACGACAGCATGGATGGGCTGCGGATGAGTTCGATCACCTACC	57
AY341249	ISCR4	DNA-Mobility	Insertion sequence common region ISCR4	CTGCCATGCGGAACGATTGGTGGCGTTCTCGTGCAAGAAG	44
AY114142	ISCR6	DNA-Mobility	Insertion sequence common region ISCR6	GAACTTCCTATACCCTTCTCCTGTACCTGCGAATTGTGCCATGCTGATACGTCGAAA	40
AJ250371	ISCR7	DNA-Mobility	Insertion sequence common region ISCR7	TGAACGAGCATGTGCATTTCCATTGCTGTGTCATCGACGG	47
AF028594	ISCR8	DNA-Mobility	Insertion sequence common region ISCR8	GCAAAGAGGGAAGCAAACTGGTGTACCGCTGTGCCAAACA	40
AF106956	rep_iterons_FIA	DNA-Mobility	F Plasmid pmk115, mini F plasmid replication origin, IncFIA replicon	CATGACGGTATCTGCGAGATCCATGTTGCTAAATATGCTGAAATATTCGGATTGACC	40
AE008868	rep_iterons_HI2	DNA-Mobility	Plasmid R478, incobatibility group HI2 (IncHI2 supgroup)	GACTTAATAGGCTCACTACCGTTGTCATCCTGTAAGTTAAGAGGTTGATCTGCTCAA	57
AF106566	rep_iterons_P IncP	DNA-Mobility	Plasmid RK2, transposon insertionsite (Tn 1723) plasmid incompatibility group	CACAGATGATGTGGACAAGCCTGGGGATAAGTGCCCTGCGGTATTGACACTTGAGGG	57
K03089	leuO	Pathogenicity	Transcription regulator, component of ilvIH-leuO-leuABCP promotor relay region	GCTGGGCGTGATAAAGGGCATCAATGGATGGAAGATTTATTAGTCTCTGTTTGTAAGCGATA	57
AF106566	lpfD	Fimbrial	Long polar fimbrial operon protein	GGTGGAACCTATGCGATGTCCTGTGAATGCCCTGATGATACCTCTCTTATAAATGAC	62
AE008875	marT	Pathogenicity	SPI-3 encoded putative transcriptional regulator	AAAGTGTGGGAAGAAGACGGCATGGTGGTGTCGGCAAATACGCTTTATCAGAATATC	57
AF161317	merA	Resistance	Hg(II) reductase: Mercury resistance	ATGAGCACTCTCAAAATCACCGGCATGACTTGCGACTCGTGCGCAGTGCATGTCAAG	57
AE008725	mgtC	Pathogenicity	SPI-3 encoded putative transcriptional regulator	CAGTGGTTACTGAATATCGTAAAAGAGGCCGCGATCTGTTTACAAGGGTTAGGTTCG	57
AE008799	misL	Pathogenicity	SPI-3 encoded protein	AACCGATTATTCCTGATCCAGTAGACCCTGTTATCCCTGACCCTGTCGTTCCCGATC	57
NC_003197	mntH	Metabolism	NRAMP manganese transport protein	GATAATTGTCGTGCTGGTCGTCGCCCTGAATATCTGGTTGCTGGTTGGAACGGTGAT	57
AE008800	mrdA	Metabolism	Cell elongation specific transpeptidase of PBP 2	GATCGCCTGTCAGAATGGATGGGTAAGTTCGGCTATGGTCACTACACCGGAATTGAT	57
X60666	msgA	Pathogenicity	SsrB-regulated factor	GATATCATTCTGGGAGAATTGACTAAGAGGGTTCACCGGATCTTCCCCGATGCTGAT	57
X60665	nanH	Resistance	Fels-1 encoded Neuraminidases	TGGTCATTGCCATCAGGATACTGTGAGGGTTTTGGTTCAGAAAATAACATCATAGAA	57
AF378725	oafA	Metabolism	Acetylation of the O5-antigen	GCACATCTTATGCCAGGGCTGAAATCGGTATTTGGAAATTCACTTAACATTACGCAGAGAAC	57
AE008831	orf 17.4	Serotyping	β- O-polysaccharide polymerase: Serogroup E_1 and E_4	TCACTTTATCACTAAATGGTCCGTTCCTGTCTACATTGCATTAGGTTTGCTACTGCCTCT	62
AE016845	orf 9.6	Serotyping	α- O-polysaccharide polymerase: Serogroup E_1	AGGTGGGCAGTTTATATTCACAGAGGCTTTTCATTCACTTGGTTATGTCGGAGTATTCCTG	60
AE008792	orf84	Phages	Putative prophage Cro protein	CCGCCTCATGTGAACGATAGCAGAACCATATTAGCGAAGGTGAACAACCATGATCAC	61
AF013776	orgA	Pathogenicity	SPI-1 encoded oxygen-regulated invasion protein	TTCACCTGAACAATTTGTTGAAACGGCAGTAGGCGTCATTAAGCATCATCTTGATGAACTTC	57
AE008783	oxyR	Pathogenicity	Hydrogen peroxide-inducible regulon activator	GCTGCATATTGGTTTAATCCCAACAATTGGTCCCTATTTGCTGCCGCTTATCATTCC	62
AF250878	pagA(udg)	Metabolism	UDG-glucose/GDP-mannose dehydrogenase	GGTGGCGTTAGACATTGTTCCTTCCCGTGTTGAACTGTTAAATGATCGGATATCTCC	57
AB041905	pagJ	Pathogenicity	Gifsy-3 encoded, PhoP-activated gene	GGCTTTAATATTACCATCCTCACTATATCTTGCTCTTACAATAGCGGCAGACTCCCAA	57
AE008892	pagK	Pathogenicity	PhoPQ-activated protein	TTTGGCCTGCTGGCATACCCTTCCCTGAAGATTGGTTTAAAATGTGTAGAGGTTATTG	58
AE008753	parA-parB IncHI1	DNA-Mobility	Putative partition protein on antibiotic resistance plasmid	GTTCCCGATATGCTGCTCATCACTCATACATTACCCCCTTGCTTCACTGAAAAACTC	59
AE008753	pefA	Fimbrial	Virulence plasmid encoded fimbrial protein	AACCAGGTTGTTCAGTTAGGTACTGTTCAGGCAGGTCAGGAAGGTACGGCTGTTGATT	57
NC_006431	pflD	Metabolism	Putative pyruvate formate lyase II	ATGACCCATCGTATTCAACGCCTCAAAGCCGCTCTGTTTCAGAATCACCGTGAAATT	58
NC_004631	phoP	Pathogenicity	Response regulator in two-component regulator system with phoQ	GAATACACCATTATGGAAACGCTTATCCGTAACAACGGTAAAGTGGTCAGCAAAGATTCGC	57
AE008747	phoQ	Pathogenicity	Sensory kinase protein in two-component regulatory system with PhoP	TGATGGGCAACGTACTGGACAACGCTTGTAAATATTGTCTGGAGTTTGTCGAGATTTC	61

AY532917	pilR	Pathogenicity	SPI-7, nucleotide-binding protein, putative sigma 54 interaction protein	AATGTGGTTATGCCTTCCCCTCAAAAGAAGCCGTGAACTATCTCTCTATGGTCAGTG	58
NC_003197	pilV	Pathogenicity	SPI-7, prepilin peptidase	CAAACCAATCGTTTCACTCAGGCAGTCTCATCCTATGTTGGAAAGTTTTATCCGACG	57
NC_003197	pipA	Pathogenicity	SPI-5 encoded protein	AGAAGGCAGGAAAGTTATTTGTCTCAATCTGGACGATTCTGATGATTCATATACCGAACA	57
U66901	pipB2	Pathogenicity	T3SS translocated protein, chromosomal coded	GCTGGACAAGTTATTGTACGAGTCAGTAAAGGCGACCATTCTGAGACAAGAGAAATTCCG	60
AF261825	pipD	Pathogenicity	SPI-5 encoded protein	CAAACAGCTAAGCAGCAGTATAAGATGGAGCAGAGCTATCTGAGATTATATGCGTCG	57
AY906856	prgH	Pathogenicity	SPI-1, needle complex inner membrane protein	AAAGAGAAGACGATAACAAGCCCAGGGCCATACATAGTTCGATTACTTAACAGCTCA	57
AM234698	Prot6E	Fimbrial	*Salmonella* enteritidis fimbrial biosynthesis protein	TGTGGGTCGTAACGCACAAGTGAAATTTACCCTGAGGGAGGCTTATGGTAATAATAATTTGG	57
AM234722	qacEΔ1	Resistance	Qac multidrug exporter, encoding Et-Br and quaternary ammonium resistance	GCAATAGTTGGCGAAGTAATCGCAACATCCGCATTAAAATCTAGCGAGGGCTTTACTAA	62
NC_003197	qnrA	Resistance	Confers quinolone resistance, plasmid located	ATGGATATTATTGATAAAGTTTTTCAGCAAGAGGATTTCTCACGCCAGGATTTGAGC	59
AE006471	qnrB2_B1	Resistance	Confers quinolone resistance, recognises both *qnrB1* and *qnrB2*, plasmid located	CAGCAAACTTCACACATTGCGATCTGACCAATTCGGAGTTGGGTGACTTAGATATTC	57
AE008837	qnrS	Resistance	Confers quinolone resistance, plasmid located	ATGGAGAGGGTTTGTTTAGAAAAATGTGAGTTGTTTGAAAATCGCTGGATAGGAACG	57
J01724	ratB	Pathogenicity	Putative outer membrane proteine	CTATTTATCTAAATCAACCTCAAGCGGCGGCATGTATTGCGGCGTAGATGAGAATAC	57
BX664015	rck	Pathogenicity	Resistance to complement killing protein, encoded on pSLT plasmid	GTACAGTTTAATCCGGTGGAAAATGTGGTCATCGATCTGGGCTATGAGGGAAGTAAAGT	59
M20134	recC	DNA-Mobility	DNA replication protein	CTCATGGGGAAAACTGGGGCGGGATTATATCTACCTGCTATCTGAACTGGAAAACAG	61
Y00768	rep_ori_g IncX	DNA-Mobility	IncX replicon - plasmid incompatibility group	GTTAGCCATGAGGGTTTAGTTCGTTAAACATGAGAGCTTAGTACGTTAAACATGAGAGC	57
M20413	rep_SG1	Pathogenicity	Replication protein encoded by SGI 1	TTCAAGAGTAAATGGATCTGAGTTTGAGAGGCGTTGTGGTGCTGCTGGTCTATACAT	59
M28718	rep_W	DNA-Mobility	Plasmid R388, class 1 integron In3, plasmid incompatibility group IncW	GTCTTGATGATCTCGTTGATACGATGGCCGGGGGCTTTGTTGTTCTTAGGCATGTTG	57
M93063	repA_A/C	DNA-Mobility	RepliconA, plasmid incompatibility group	GGACCACCAGCTAGAAAGTATTAACGGAACAATCATGAGTAAGAGAACCAAAGACAAA	57
AF261825	repA_B_C IncL/M	DNA-Mobility	Plasmid pMU407.1, plasmid incompatibility group, Inc L/M	TATACTCAGGTGGTTATAGTCGTTTGTAGTCGGAGGGCTTGTGAGCAGAGTAGTTGAG	58
U12441	repA_FIB	DNA-Mobility	IncF plasimid RepFIB replicon, plasmid incompatibility group	GAAGTAAGTTAATGACATAAACTATGGTCAGTATGCCAGACTCAGTTGTTAAATACAGGCTGC	58
X73674	repA_FIIs	DNA-Mobility	pSLT, plasmid incompatibility group	CATTATGATCCACTGGCCAACCGTGTACAGAGATCCATCACCAATCTGGCTATAGAG	63
U27345	repA_N	DNA-Mobility	IncN plasmid R64, plasmid incompatibility group	TATCTGGGAAATCGAATTTAACCATAAACTCCTGCGGTACATTTACGGCCTGACGAA	57
M26308	repA_repB IncF	DNA-Mobility	Plasmid pRK100, plasmid incompatibility group	GTACTGCGAGAGAGAGGGGATAACACAGGCTCAGTTCGTTGAGAAAATCATCAAAGAT	57
AE006471	repA_T	DNA-Mobility	Plasmid Rts1, plasmid incompatibility group IncT	TAATCAAAGTAGTATAACTCCCATAATCGCTCGTCTGCTTCCAGTTCCACAACGTCTGTATCG	58
NC_003292	repA_Y	DNA-Mobility	IncY plasmid P1, plasmid incompatibility group	TCTTTACGCAGACATTGAAAGTAAGGCAAAAGAACTAACAGTTAATTCAAACAACACT	63
AY234375	repA2_FIC	DNA-Mobility	Plasmid F, putative repliation protein genes	ATCTTCACATTGATTCCAGCAAGTATCCTCACCCGTTTTGCAGCCTTCTCCAGAAAA	58
K00053	repC_DT193	DNA-Mobility	DNA replication protein	AAGCACATACAGGACTGCATCGAGCGCCTTTGGAAGGTATCCATCATCGCCCAGAAT	57
K02380	repC_R64	DNA-Mobility	DNA replication protein of plasmid R64	GGAAAATACTTCGGTCAACGCCCTAGCCGAACGCTTCCTCGATGACGGCCTGAAAAC	57
M16168	rfbD	Serotyping	TDP-dehydrorhamnose synthetase: Serogroup A,B,C_2-C_3,D_1,D_2	TGACCTTATTCTGCCTCAATGGGAATTAGGAGTTAAGCGTATGCTGACTGAAATGTT	57
AY524415	rfbE	Serotyping	CDP-tyvelose-2-epimerase: Serogroup A and D	ACAGTGGTGTTCAGGCATTCATCAATGTATGGTGGGAGACAGTTTGCTACTTATGATC	60
AP005147	rhaA	Metabolism	L-rhamnose isomerase	GTCTGATCCCAGGCCCAAAACGGCTGAACCTACACGCCATTTACCTTGAGTCGGATA	57
AE008792	rhuM	Pathogenicity	SPI-3 encoded putative cytoplasmic protein	GAAGCCGAAGGTGAGAAGGATATCGCCGGTTTGCTACAATGGGAAACAGAACCTAAA	58
AL627273	rep_RNAI IncI1	DNA-Mobility	Plasmid incl-1 minireplicon, plasmid incompatibility group	CATAAGCGACAGCTTGTGGCAGGTCTGAAGAATACTCCATATAACGCAGTACACTGG	57
AE008889	rep_RNAI_BO	DNA-Mobility	Plasmid pMU720, IncB minireplicon encoding RNA I, RNA II and Promotor regions	TCACATAAGGATGTATCTGTGGCAAGAGCGAAGATAAGCAGTTGAATAGATCGTTATATT	57
AE008874	rep_RNAI_K/B	DNA-Mobility	Plasmid R387, replication-associated protein, plasmid incompatibility group	CAGCTTGTGGCAGGTCTGAAGAATACTTCATATAACGCAGTACACTGGAGTCAGTTAGC	57
AE008833	rpoS	Metabolism	Major sigma factor during stationary phase	GTCTGCTGGGATATGAAGCTGCGACACTGGAAGATGTAGGCCGTGAAATCGGTCTTA	60
AE008708	safC	Fimbrial	Putative fimbriae usher protein	GTAAGTGCTAGTTGGCAGATGACTTCACCATCACACGGTGGTCAGACGCAACAAGTG	59
M63169	sat (Tn7)	Resistance	Streptothricin acetyltransferase of Tn7	AAGAGCTTGTCGGGAAGATTGAACTCAACTCAACATGGAACGATCTAGCCTCTATCG	57
AB161461	sat1 (int2)	Resistance	Streptothricin acetyltransferase linked to integrase 2	CAAGCTATGAGCCAGGTCAAGCTCCATATTCCGTTGAAGAATTAGCAGATGATGTGG	57
NC_004313	SB10	Phages	Encoded by ST64B Phage	GTTATGTGCTCTCCCGCGAATTGCGAAAATCACTCCGTCCTCAGCGAAGGAAGAAAA	57

NC_004313	SB54	Phages	Encoded by ST64B Phage	TTGAACTACTGGCAATCAGATATGCAGCATGGATTAAGCCGGAATTTGAAATCGAAG	57
L11008	sefA	Fimbrial	Fimbrial protein encoded by *S*. Enteritidis, *S*. Dublin and *S*. Gallinarum	TTTCCGTGGGCGTATTCAGGGAGCCAATATTAATGACCAAGCAAATACTGGAATTGA	57
AF239978	sefR	Fimbrial	Sef14 fimbrial regulator	AGCCGTTGTGGAAATTGAATATATGGATGACATTGAATCATTTGACATTATTACTTTGCCAGA	57
Xxxxxx[1]	SEN4287	Metabolism	Putative restricition endonuclease gene *Salmonella* Enteritidis unique PT4	GTCGGTAGTGATTAGCGTACCGCGAATGGCTTTGTGGTAGGGTAGTGCACCGCGTAG	57
AE008719	sfbA	Pathogenicity	Putative periplasmic iron-binding lipoprotein	CGACGCCAACCTCTTTCAACATACCCTCTATTTTGACAAATTCACCGCTGACAAAGG	63
NC_003197	sgbE	Metabolism	Ribulose-5-phosphate 4-epimerase	GAAATTAACGGCGAGTACGAGTATCAGACCGGCGAGGTGATTATTAAAACCTTTGAA	57
NC_003197	shdA	Pathogenicity	Fecal shedding factor	CAATATGGTTTCTACAACAACAGCGTAGAGAGCGGTGATGCGGGATCTGAATCTTAT	57
U51867	sifA	Pathogenicity	Lysosomal glycoprotein (lgp)-containing structures	CAAGAAAAGGCAACCTACCTGGCAGCGAAAATTCAGTCTGGGATTGAAAAGACAACG	57
AE008831	sipA	Pathogenicity	SPI-1, *Salmonella* (cell) invasion protein	GCAGTAACCATAGCGTGGATAACAGTAAGCATATTAACAATAGCCGAAGCCATGTCG	57
AF026035	sirA	Pathogenicity	Invasol SirA: Regulator of invasion proteins	GTAAAGGTGGGGTACGTAATGATGACACATATCCGAATGCCAGTAACAATGCCGAAG	57
AF128999	sitA	Pathogenicity	Invasion SirA: Regulator of invasion proteins	GATATTAAACGAGCGCAGGGGGCACAGCTTATCCTCGCGAATGGTCTGAACCTGGAG	57
AF127079	slrP	Pathogenicity	Leucine-rich repeat protein	TATGATAACAGCATAAGGACACTGCCAGCACATCTTCCGTCAGAGATTACCCATTTGAATG	61
AE008762	slyA	Pathogenicity	MarR family transcriptional regulator for hemolysin	GAGCTTCTGATTAAACTTATCGCCAAACTTGAACACAATATTATGGAATTGCACTCTCACGA	62
AF007380	sodC1	Pathogenicity	Gifsy-2 encoded, copper/zinc superoxide dismutase	TATCCGTTACTGGCACCACGCCTTAAATCACTGTCAGAACTGAAAGGTCACTCATTG	57
AF254764	sodCIII	Pathogenicity	Fels-1 encoded putative Cu/Zn superoxid dismutase precursor	TATTACAATTACCGAAACAGAATATGGCTTGTTATTCACCCCACATTTGTCCTCACTTCC	60
AF121227	sopA	Pathogenicity	Secreted outer protein	CAGATAATTTCCTGCTGCTTTCCTCCCAAGATTCAGACACGGCGATGATGCTCTCCA	57
AE008747	sopB	Pathogenicity	SPI-5 invasion gene D protein	CGGCAAAGATCGTACAGGGATGATGGATTCAGAAATCAAGCGAGAGATCATTTCCTTACATC	62
AE008834	sopD	Pathogenicity	Secreted outer protein	TGCCCGGCTCATCAAGATCTGTTTACTATCAAGATGGACGCTTCTCAGACACAATTT	57
AL627268	sopD2	Pathogenicity	Secreted effector protein, sopD homolog (pseudogene)	TGGAATGTGTTGAATGGAATGGTACGCTTACTGAAGAGGAGATGAATAAACTACGCTGTCT	63
L78932	sopE1	Pathogenicity	Translocated effector protein, encoded by P2-like cryptic bacteriophage	ACGTTTTATTTCGCATAAGAACACTGAATCTTCTGCAACACACTTTCACCGAGGAAGC	57
AF200952	sopE2	Pathogenicity	Secreted outer protein	GTGACTAACATAACACTATCCACCCAGCACTACAGAATCCATAGAAGTGACGTTGAACCAGT	61
AE008832	spaS	Pathogenicity	SPI-1 encoded surface presentation antigen	ATATTGTAGGTATTGCCGTCATTTGGCGTGAACTTCTCCTCGCATTGGTATTAACTTGC	58
AF060869	siiD (spi4_D)	Pathogenicity	SPI-4 HlyD family secretion protein, predicted cation efflux pump	ATCTCTTTCTAAAGGAGGGACGATACAAGATATTTATGTAGCCGAGGGTGATACTGT	62
AF060869	siiE (spi4_F)	Pathogenicity	SPI4 encoded protein	TAATGGTATTGCTGTCGGTCAGGCTGTAACGGATAGTTTGGGTAACTTCACCTTTAC	59
AF060869	siiF (spi4_R)	Pathogenicity	SPI-4 encoded putative ABC-type bacteriocin/lantibiotic exporter	GGGCGTGAGTTATCAGTATGATGCTCAATCTCCGATGATTATTAACCGACTGTCTAT	57
NC_003197	sprB	Pathogenicity	SPI-1 encoded transcriptional regulator for Type III Secretion System (TTSS)	TGTGTGCTGCAATATTTTGGCGTTATGGATTATGTTTTAAAAGACGAACTATCCTGCG	57
AE008831	sptP	Pathogenicity	SPI-1 encoded Protein tyrosine phosphatase	ACCTTATTAAGAGCAAGGATAATGTTGGTGTCAGGAATGCCGCTTTAGTCATAAAAGGC	57
AE006471	spvC_a	Pathogenicity	*Salmonella* plasmid virulence: hydrophilic protein	GGTTACGATGTTTTCATCCATGCTCGTCGAGAATCACCTCAGTCTCAGGGCAAATTT	58
AE006471	spvC_b	Pathogenicity	*Salmonella* plasmid virulence: hydrophilic protein	CGGTGACAAGTTCCACATCAGTGTGCTCAGGGATATGGTGCCACAAGCATTTCAAGC	59
AE006471	spvR	Pathogenicity	*Salmonella* plasmid virulence: regulation of spv operon, lysR family	ACAAAGACTCTTTATACGGAAGAATGGCACTCTTATCCCAACCGAATTTGCACAAACT	57
AE008907	srfJ	Pathogenicity	putative virulence factor, activated by transcription factor SsrB	TGACATGATTGGTAATTTCAAATCGGGTTGTAGCGGGTTTATCGACTGGAATCTGCTG	57
AE008761	ssaQ	Pathogenicity	SPI-2 encoded secretion system apparatus protein	ATACCACAACAGGTGCTCTTTGAGGTCGGACGTGCGAGTCTGGAAATTGGACAATTA	58
NC_003197	sseC	Pathogenicity	SPI-2 encoded translocation machinery component, required for systemic infection	AAATAGAGCAATTAATAACTCAGCAACGGTTTCTGGATTTCATAATGCAACAAACAGA	58
AE008761	sseF	Pathogenicity	SPI-2 encoded secretion system effector	ATTCATATTCCGTCAGCGGCAAGTAATATAGTCGATGGTAATAGTCCTCCTTCCGATATA	57
AE008743	sseI	Pathogenicity	Gifsy-2 prophage putative type III secreted protein	GCAACAGAACCGGGAGTGGAACGCACAGATATAACTTACAACCTAACCAGTGATATTGAT	58
AF294582	sseJ	Pathogenicity	SPI-2 regulated translocated effector protein,	CACATCATATCTTACCCTCCTATGGTCAATACTTTGGCGGAAGGTTTACTAATGGATTTACC	60
AE008894	sseK1	Pathogenicity	SPI-2 encoded T3SS *Salmonella* secreted effector K1	GGGATAATAGCTGTTGATCGCAATAACCACCCGGCTTTACTTGCTGGATTAGAAATAATGC	60
AE008795	sseK2	Pathogenicity	*Salmonella* secreted effektor K1, putative SPI-2 T3SS	TTTCATGTCAAAGTAATACTCAAACCATCGCACCTACGCTCAGTCCACCTTCATCAG	62
AF013776	sspH1	Pathogenicity	Gifsy-3 encoded leucine-rich repeat protein, S*almonella* secreted protein H1	CTTACCTTCCCCGCGTGGGAGGAGAATATTCAGTGTAACAGGGATGGTATAAATCAG	61
AE008800	sspH2	Pathogenicity	Leucine reach repeat protein, *Salmonella* secreted protein H2	GATGTCTTCCCGCCACCATCAGTAATCGCCGCATTTATCGTATTGCCTGGTCTGATA	57

AE008761	ssrB	Pathogenicity	SPI-2 encoded protein: Secretion system regulator,	CCTGTTGTGCATACGAGCCTGACATACTTATCCTTGATCTTAGTCTACCTGGCATCA	57
AL627265	staA	Fimbrial	Fimbrial protein encoded by *S*. Typhi CT18	CGGCTGATGTAACTGATGCCACTAAGGCTTCTCTGGTAATGGATTCGTCATTCTCTA	57
AE008710	stbD	Fimbrial	Fimbial usher protein	AATATCGGTTTGCCAACGGTGATTAGCGTCAGTAATAGTGAAACATTTCAACCCTCCG	58
AE008795	stcC	Fimbrial	Paral putative outer membrane protein	TGTAGTAGATCATCATGGTCATAATGTGGGCATTGTTGGACAAGGTAGTCAGCTATTTATTCG	63
AE008839	stdB	Fimbrial	Putative outer membrane usher protein	AATTACTGGAACGCACAGTCCAACAATAACTACATGCTCAGCCTCAACAAGGTGTTC	57
AL627276	steB	Fimbrial	Outer membrane fimbrial usher protein encoded by S. Typhi CT18	CAGCCCGGATCAGAGTAACTATAACCTGTCTCTTTCCTGGTACTTCGACTTAGGGTC	57
AE008703	stfE	Fimbrial	Putative minor fimbrial subunit	GGCGGTGAGGTGGAATTTGGCAATGTGTTGACGACGAAAGTGGATGGGGTGAATTAC	57
AL627280	stgA	Fimbrial	Fimbrial protein encoded by S. Typhi CT18	GATTCTGCGTATAGCACGATTGATACCACAGCGGGTACGGCTTCTATGGAGTTTATC	57
AE008702	stiC	Fimbrial	Putativie fimbrial usher protein	TGAACTACAGCTTCAGCGGCTATAAAGAGTACGGTTCCAGTGAGGATTCCGACGATG	57
AE008915	stjB	Fimbrial	Putative fimbrial usher protein	GGTTATTACACTTATCAGGCTACGGATAATGACAACGACTCTCGCAGTATAATGGCTTCCT	57
NC_006511	stkC	Pathogenicity	Outer membrane usher protein	CGGAACCTATCGTGTGAAAGTGAATCTTAACAATGCGTTGAAATCTACCTCTGAAATTACC	57
AE0098709	STM0305	Serotyping	Discrimination between *Salmonellae* subspecies I and non subspecies I	CACCTGTTCAAAGATTTTCTGAAGGCGCAGGAGTATTCATTACTGATATCCTCCATT	57
AE008710	STM0330	Metabolism	Putative 3-isopropylmalate isomerase, (dehydratase), subunit with LeuC	TGCGAAATTACGACGGCAGAGGAAACGATCTCCTTTGTGATCAGTGAACTCAAACGG	57
AE008737	STM0900	Phages	Fels-1 prophage encoded protein	GTAAGCAACTACATCAGACACAAACTTATCAGATATGCAAAAGGAAAGGGGAAGGCGGC	59
AE008784	STM1896	Metabolism	Putative cytoplasmic protein	TTTCAGTAGATGTTTCCGACAATGGTATTTCTGGCGTGGCAAAAGAGTGGCTTAAAG	57
AE008819	STM2616	Pathogenicity	Gifsy-1 prophage encoded protein	ACCACTCAAATCTCTGTCGAAACTCTTTCCCCGATTACCCATAACCAAATTCCCGTTA	59
AE008823	STM2701	Pathogenicity	Fels-2 prophage encoded protein	GAATGCTGATCTGGCCTGACTTCATCAACTTTGACACCGTGCTGAAAGCAGACGCGA	57
AE008824	STM2740	Pathogenicity	Fels-2 prophage encoded protein	CTACAGAACGCTTCTATCCGCAAGGAAACCCGCTGCCTTATCGAATGGGAGCTACTG	58
AE008842	STM3098	Serotyping	Discrimination between salmonellae and non salmonellae	GCTATGGGAAGACCAGATTATCTATATTTATCACTCTACGCCGGGTTCGCAGGGAAG	57
AE008876	STM3782	Metabolism	Putative PTS system galactitol-specific enzyme IIC component	AGGTAACCCAGCCATTATATCTACAGCACTGATTCTGACACCTATCTCTGTCTTTATTGC	60
AE008889-1	STM4057	Serotyping	Discrimination between *Salmonella* subspecies I and non subspecies I	TGATCATTACGTTGTGATTTATTCCAACGGTACGCTGTATGGGGAATGGCCC	52
AE008896	STM4200	Phages	Putative phage tail fiber protein H	TGGAGGTGGAGGGCATACGAGTAATACAGATGGTCTTCTCTATTGTTCAGGTGGTAA	57
AE008896	STM4210	Phages	Putative methyl-accepting chemotaxis prophage encoded protein	GATGAACTGCTGAGCGTCGTTGAAGAGGGTATGCGTGAAGCCAAAGAGATGATGGAT	52
AE008911	STM4497	Metabolism	Putative cytoplasmic protein	AAAAACAACGGCTCCGGTAATGAGATTGGGTTCTGGATTTTTGATTATCCTGCTCAG	57
AE008916	STM4595	Fimbrial	Putative fimbrial chaperone protein	GATTACGTTCAATGGCAAAATTTACGATCAGGCGTGTACGGTTCAGGTGAATGGCTC	57
M28829	strA	Resistance	Aminoglycoside-6"-phosphotransferase, encoding STR resistance	GAACAGCAGATCGCTATGCCGATTTGGCACTCATGATTGCTAACGCCGAAGAGAACT	57
M28829	strB	Resistance	Aminoglycoside-3"-phosphotransferase, encoding STR resistance	GGACTCCTGCAATCGTCAAGGGATTGAAACCTATAGAAGACATTGCTGATGAACTGC	57
AL627279	STY3672	Phages	Hypothetical phage protein encoded by phage cs73 of S. Typhi CT18	ACAGAAGATTCCATTACAGATGTGTTTAACCAAGTGCCTTCATCGTGTATATCCGACAGTGC	57
AL627279	STY3676	Phages	Phage protein, putative capsid scaffolding protein	CTGATTAACCTTGAGCACATCAAGTCTTATCTGCCGGACAGCACCTTTAACCGCTAC	57
AL627281	STY4221_1	Metabolism	Putative aminotransferase	ATTAATGGATTTCCAGTAATACCTATGTCTGTTGCAGATACCGATTTAGTTTCTCCCGTGG	62
AL627283	STY4625	Phages	Phage protein, major capsid in phage P2, homologue with Fels2 protein	CGATCCGACGCTGATGGAAGACGTGGAATACAAATGCGAGCAGACCAACTTTGATAC	57
AL627283	STY4631	Phages	Hypothetical phage protein encoded by phage cs73 of S. Typhi CT18	ACATAAGCCTGAAGGTAAGAAGCAGAAGCACACGCATCCTGTAGATGTTGTATTTAGT	61
AF106566	sugR	Pathogenicity	SPI-3 encoded putative ATP binding protein	CGCATTTCCACTAATCCAGTTTATTGTCACTACCCATAGCCCGCAGGTTATCAGCAC	57
X12869	sul1	Resistance	Dihydropteroate synthase encoding SUL resistance	CTCTTAGACGCCCTGTCCGATCAGATGCACCGTGTTTCAATCGACAGCTTCCAACCG	58
M36657	sul2	Resistance	Dihydropteroate synthase encoding SUL resistance	TTCTATCCGCAATTGGCGAAATCATCTGCCAAACTCGTCGTTATGCATTCGGTGCAA	57
AJ459418	sul3	Resistance	Dihydropteroate synthase encoding SUL resistance	AAATAACTGGAACCGATGTGAAATCTCGTTTAGCACCAACTCTTGCAGCAGAAATGTATGC	57
AL627266	tcfA	Fimbrial	Typhi colonisation factor, putative fimbrial protein	GTATGCCACAGGAGAAGGAGGTACCAGCAGGGAATGATATAGAGACAGGACTTGTTG	57
X61367	tet(A)	Resistance	Efflux pump, encoding TET resistance	ATCGTCGGACCCCTCCTCTTCACGGCGATCTATGCGGCTTCTATAACAACGTGGAAC	61
V00611	tet(B)	Resistance	Efflux pump, encoding TET resistance	TTGGATGGAATAGCATGATGGTTGGCTTTTCATTAGCGGGTCTTGGTCTTTTACACT	57
J01749	tet(C)	Resistance	Efflux pump, encoding TET resistance	CATGACTATCGTCGCCGCACTTATGACTGTCTTCTTTATCATGCAACTCGTAGGACA	57

L06798	tet(D)	Resistance	Efflux pump, encoding TET resistance	CGGAGCAGAAACAAGAAAGCGCAGGTATCAGCTTTATCACACTGCTTAAACCTCTGG	57
L06940	tet(E)	Resistance	Efflux pump, encoding TET resistance	CGGCGTTATTACGGGAGTTTGTTGGAAAGGCTAATGTTGCAGAGAACTACGGTGTTT	57
S52437	tet(G)	Resistance	Efflux pump, encoding TET resistance	GCCTCACCAATCTAAGCTCTATCGCAGGACCGCTTGGCTTCACAGCACTCTATTCTG	57
NC_006816	tnp_Cf	DNA-Mobility	Transposase insertion sequence IS	CATCGACCAGTGCTTGGATCTCAGTGATCTACGTGCCTACCTGGCAGATTTCTATAG	57
NC_006816	tnp_IS1	DNA-Mobility	Transposase insertion sequence IS1	GGTGGAGCTGCATGACAAAGTCATCGGGCATTATCTGAACATAAAACACTATCAATA	57
NC_002056	tnp_IS102	DNA-Mobility	Transposase insertion sequence IS102	GTGAATATGCAGACCGTAACCGTGCAGTGGCTAATCAGCGAATGACCGGGAGTAATG	57
NC_003384	tnp_IS1202	DNA-Mobility	Transposase insertion sequence IS1202	CTATTTTGAAGCGACCCGTGGCTATATCGAGAAATACGGTAAGCCCATGATCCTTTA	57
NC_006816	tnp_IS1294	DNA-Mobility	Transposase insertion sequence IS1294	TTTTAAGATGGTGAGGTACTTCGGGTTCCTTGCCAACCGTGTGTGTGGAGAGAAGCT	57
NC_003198	tnp_IS1351-like	DNA-Mobility	Transposase insertion sequence IS1351like	GAATCACAAAAGGCTCCACCGTATTTACTGTCTGCTCAAGCTGAATTTTCGCCGTAA	57
NC_002305	tnp_IS30	DNA-Mobility	Transposase insertion sequence IS30	GCTAAACAACAGACCGAGAAAGACACTGAAGTTCAAAACACCGAAAGAGATAATTGA	57
AJ310778	tnp_Orf341E	DNA-Mobility	Transposase Orf341E	CTCAATGTCCACTACCACATGCTGTTTCTCGATGGTGTCTATGCCGAAGATGACTAT	57
AJ634602	tnp_pFPTB1	DNA-Mobility	Transposase insertion sequence on Plasmid pFPTB1	TACTTTGGTAATAACAGAGGGATCACCTGGTACAACTTTGTGTCCGATCAGTATTCC	57
NC_006511	tnp_SPA2465	DNA-Mobility	Transposase SPA2465	GCTGAATGAGGTGCGGGAAATTACGGATAAAGGGTTATCAGAATATAACTGCGAACG	57
NC_003198	tnp_STY343	DNA-Mobility	Transposase STY343	CAGATCATCGCTGTGATTAGATCAGTTGAATCCGGACGGACTGTTAAAGATGTCTAC	57
NC_003384	tnp_Tn2680	DNA-Mobility	Transposase of Tn2680	CTTTGAATGGGTTCATGTGCAGCTCCATCAGCAAAAGGGGATGATAAGTTTATCACC	57
NC_003384	tnpA_IS1-like	DNA-Mobility	Transposase A of insertion sequence IS1like	TGCAGTTCACTTACACCGCTTCTCAACCCGGTACGCACCAGAAAATCATTGATATGG	57
AJ746361	tnpA_IS1696	DNA-Mobility	Transposase A of insertion sequence IS1696	TCATATCAACCCGAAGTATGGAAGCAGTCCAGGACGGACTTTCTACACCCATATCTC	57
NC_006816	tnpA_IS186	DNA-Mobility	Transposase A of insertion sequence IS186	CGCTGAATGGCGACTACATATGGGATATGATCCTCATACCTGTCAGTTCACTGATTT	57
AY341107	tnpA_IS200	DNA-Mobility	Transposase A of insertion sequence IS200	AAATACCGAAGACAAGCGTTCTATGGAGAGAAGCGTAGGGCAGTAGGCAGCATATTAA	57
NC_006816	tnpA_IS26	DNA-Mobility	Transposase A of insertion sequence IS26	TTGAACACCGACAGATTAAGTACCGGAACAACGTGATTGAATGCGATCATGGCAAAC	57
AY509004	tnpA_IS3/IS911-like	DNA-Mobility	Transposase A of insertion sequence IS3/IS911 like	GAGGAAGAGAACACCAGACTCAAGAAGCTGCTTGCCGAAGCCATGCTGGATAAAGAG	57
AY509004	tnpA_IS4	DNA-Mobility	Transposase A of insertion sequence IS4	AAGTTTGATTCCTTGGACTCTTCAGAATACAGACAGCAAATAAAGACCTTTCGTTTGA	57
AY509004	tnpA_IS406	DNA-Mobility	Transposase A of insertion sequence IS406	AAATGTATGTCAAAGGAGTAAGTACCCGCAGGGTCTCGGATATCGTCGAAATTCTTT	57
AF261825	tnpA_IS6100	DNA-Mobility	Transposase A of IS6100, SGI 1	CCGATCACGGAAAGCTCAAGATACTGATCAAGCCGGTGCGCGGTTTCAAATCGATCC	57
AF071413	tnpA_Tn21	DNA-Mobility	Transposase A of Tn21	GACTCCAAGGACGACCTGATCCGACATTACACATTCAACGATACCGACCTCTCGATC	58
AY509004	tnpA_Tn3	DNA-Mobility	Transposase A of Tn3	GTGTTCTTCAACCGCCTTGGGGAAATCAGGGATCGGAGCTTCGAG	57
AP005147	tnpR_IS10	DNA-Mobility	Tesolvase of insertionsequence IS10	CATGGTATAAATCCGTTGAGAAGCTGGGTTGGTACTGGTTAAGTCGAGTAAGAGGAA	45
AF261825	tnpR_SG1	DNA-Mobility	SGI 1 Resolvase	AGGGAGATTAGGGCATTACTCAAAGATGGTTCTATTCCTGTATCTGATGTTGCTAGGCGA	57
AY144490	tpase1	DNA-Mobility	SPI-3, transposase1 similar to transposaseA	CGATTGTTAGGTTAAGGACACACCCTAATACCCCATTTGTTTCTGCTATCCTCAAAC	57
AE00647	traT	DNA-Mobility	pSLT plasmid encoded conjugative transfer surface exclusion	GCGATGAGCACAGCAATCAAAAAGCGTAATCTTGAAGTGAAAACCCAGATGAGTCAG	57
AF261825	trhH	Pathogenicity	SGI 1 encoded putative pilus assembly protein	ATAACAGCCTGCTTGAAGCCATGATGTCTATAACTGGTGCCGTTGTCATTGGTGATT	57
M84642	wbaA_C1	Serotyping	O-antigen-polymerase: Serogroup C_1	GTGCTTGGTGCCATTCTATCATTGCCTTTGTCACATTATTTATCAGATATAATTTCTTCCGTT	63
X60665	wbaO_E1	Serotyping	Manosyl transferase (β 1-4 linkage): Serogroup E_1 and D_2	ATCTTTGGATTTTAGGCTTGGCTCCCAGACCTTTACTGAAGTTGATCGGCAAGTGTATATC	61
X56793	wbaU_B	Serotyping	Manosyl transferase (α 1-4 linkage): Serogroup B and D_1	TGCCTGATGCAATTTCCCGATTTAACAACTATGTCGCACGGTATGACTTTGATAATATGAAGC	63
X56793	wbaV_B	Serotyping	Abequosyl transferase: Serogroup B	CGGGTGTGATTTAGTTGAGATTAGAAACCCTCATCGTTCTTGGCTCAGAACAGATGATGAAC	62
M65054	wbaV_D1	Serotyping	Tyvelosyl transferase: Serogroup D	TCGGCGATGGTTAAATGGTGGCAGTAGATTATTTTCTTTTAGCAATGAAGCTGATTTGATAGA	63
D14156	wcdA	Pathogenicity	UDP-glucose/GDP mannose dehydrogenase	GATTATTGGGCTGGGATATGTTGGGCTTCCTCTGGCAGTTGAATTTGGCAAATCTCG	57
D14156	wcdE	Pathogenicity	Required for translocation of the Vi polysaccharide to the cell surface	TACTCAAACAAGAGGATTGGGAGGGGCTATGCCTCTATTTCAGTCAGCATCCCGAAAA	58
D14156	wzf	Pathogenicity	Vi polysaccharide export inner-membrane protein	GATTCTGTCCGTAGAGCGTCATTAGTTATTAAAGAGCGACGTGTTCAGCAAGCCAAG	59
AY334017	wzx_O6,14	Serotyping	O antigen flippase: O6,14 serovar factor	AAAGCGACCTTGAGTATTGGGCTCACTGCTGTAGTAGTTATAATTTATAGTAGAGTGGA	57

AF017148	wzy(O27)	Serotyping	α- 1-6 polymerase: O27 serovar factor	TGAGTCTTTATTTAATCAAATATCTTTTATGCGGATGCTGGATTGGCTACATCAAGGGGCAGT	63
AE008758	wzy_B	Serotyping	α- 1-2 polymerase: Serogroup B	TGGCGAATTACTCGGATTATACCCGTAATGCTGTTCTTGTTGCTTCCTCAAACTTTG	57
U04165	wzy_D2/E1	Serotyping	Putative O antigen polymerase: Serogoup D2 and Serogroup E1	AGGCGGTCAGTTTATATTCACAGAGGCTTTTCATTCACTTGGTTATGTCGGAGTATTCCTG	61
AE008706	yafD	Metabolism	Putative cytoplasmic protein	TGGTAGTAAATGTTCATGCGGTAAATTTTAGTCTGGGCGTGGACGTATACAGTAAGC	57
			Control oligonucleotides		
AF282268	ttrC	pos. Control	SPI-2 encoded tetrathionate reductase subunit C	ATGACGCATTCACTCATCATTGAAGAAGTGCTGGCTCACCCGCAGGACATTAGCTGG	57
X58149	PRKase	neg. Control	PRKase of *Arabidopsis thaliana*, negative control probe	TAACCTCTTCTTCTTCTTCCAAACAAGTCTTCCTCTACCGTCGTCAACCACAAACCA	57
M86720	rca	neg. Control	RCA of *Arabidopsis thaliana*, negative control probe	GATGATGAAGTGAGGAAGTTCGTTGAGAGCCTTGGAGTTGAGAAGATCGGAAAGAGG	57
NM_121758	rcp1	neg. Control	RCP1 of *Arabidopsis thaliana*, negative control probe	AGGTGTTAGGTTTGTAGGGTCTTTATCTGGATGGACAGCAACTCTTATGTTCATGTGGATG	57

[1] Sequence of SEN4287 available from www.sanger.ac.uk/projects/salmonella

Table A3: *Salmonella* strains used in the serovar 4,12:d:- investigation study and their characteristics.

BfR-No.	Serovar	Phage type	Source	Year of isolation	Array type	PFGE[a] type	Resistance[b]
94-01172	4,12:d:-		Broiler	1994	1	5	Susceptible
98-02398	4,12:d:-		Broiler	1998	1	8	Susceptible
02-02398	4,12:d:-		Broiler	2002	ND	7	Susceptible
02-04641	4,12:d:-		Broiler	2002	ND	10	Susceptible
03-01904	4,12:d:-		Broiler	2003	ND	6	Susceptible
03-02117	4,12:d:-		Broiler	2003	1	2	Susceptible
03-03421	4,12:d:-		Broiler	2003	ND	1	Susceptible
04-00176	4,12:d:-		Broiler	2004	ND	1	Susceptible
04-00981	4,12:d:-		Broiler	2004	ND	6	Susceptible
04-02236	4,12:d:-		Broiler	2004	ND	3	Susceptible
04-02830	4,12:d:-		Broiler	2004	1	1	Susceptible
05-04891	4,12:d:-		Broiler	2005	1	1	Susceptible
05-05163	4,12:d:-		Broiler	2005	ND	1	Susceptible
06-01203	4,12:d:-		Broiler	2006	1	1	Susceptible
06-01260	4,12:d:-		Broiler	2006	ND	1	Susceptible
06-01480	4,12:d:-		Broiler	2006	ND	1	Susceptible
06-01642	4,12:d:-		Broiler	2006	1	1	Susceptible
06-04044	4,12:d:-		Broiler	2006	ND	1	Susceptible
07-01001	4,12:d:-		Broiler	2007	ND	1	Susceptible
07-02442	4,12:d:-		Broiler	2007	ND	1	Susceptible
07-04661	4,12:d:-		Broiler	2007	ND	3	Susceptible
08-01120	4,12:d:-		Broiler	2008	ND	1a	Susceptible
08-01322	4,12:d:-		Broiler	2008	ND	3	Susceptible
08-01574	4,12:d:-		Broiler	2008	ND	1	Susceptible
02-02394	4,12:d:-		Broiler	2002	ND	2	Susceptible
04-00098	4,12:d:-		Feed	2004	2	9	Susceptible
05-04943	4,12:d:-		Feed	2005	1	1	Susceptible
06-01412	4,12:d:-		Feed	2006	2	1	Susceptible
07-00780	4,12:d:-		Feed	2007	ND	6	Susceptible
07-01353	4,12:d:-		Feed	2007	ND	1	Susceptible

07-01533	4,12:d:-	Feed	2007	1	1	Susceptible
08-04702	4,12:d:-	Human	2000	ND	5	Susceptible
08-04696	4,12:d:-	Human	2001	ND	4a	Susceptible
08-04701	4,12:d:-	Human	2001	ND	4	Susceptible
07-02168	4,12:d:-	Human	2002	1	4	Susceptible
07-02169	4,12:d:-	Human	2002	1	2	Susceptible
07-02171	4,12:d:-	Human	2002	1	2	Susceptible
08-04694	4,12:d:-	Human	2002	ND	2	Susceptible
08-04695	4,12:d:-	Human	2002	ND	4	Susceptible
08-04699	4,12:d:-	Human	2002	ND	2a	Susceptible
08-04700	4,12:d:-	Human	2002	ND	2	Susceptible
07-02170	4,12:d:-	Human	2003	1	2	Susceptible
08-04698	4,12:d:-	Human	2003	ND	4	Susceptible
07-02167	4,12:d:-	Human	2004	1	3	Susceptible
07-02166	4,12:d:-	Human	2005	1	2	Susceptible
08-04703	4,12:d:-	Human	2005	ND	1	Susceptible
08-04697	4,12:d:-	Human	2006	ND	1	Susceptible
07-02165	4,12:d:-	Human	2007	1	3	Susceptible
04-01557	4,12:d:-	Pig	2004	1	5	Susceptible
04-02779	4,12:d:-	Pig	2004	ND	5	Susceptlible
05-03032	4,12:d:-	Pig	2005	ND	2	Susceptlible
98-01070	4,12:d:-	Turkey	1998	1	7	Susceptible
02-00476	4,12:d:-	Turkey	2002	ND	4	Susceptible
03-01731	4,12:d:-	Turkey	2003	ND	6	Susceptible
07-02602	4,12:d:-	Turkey	2007	ND	1a	Susceptible
04-00787	4,12:d:-	Turkey	2004	1	3	Susceptible
05-04338	Schwarzengrund[c]	Turkey	2005			Susceptible
04-02395	Stanley[d]	Pig	2004			Susceptible
05-03027	Stanley	Pig	2005			Susceptible
03-03327	Duisburg[e]	Broiler	2003			Susceptible
03-03328	Duisburg	Broiler	2003			Susceptible
07-02558	Derby[f]	Human	2007			Susceptible
07-02554	Derby	Pig	2006			AMP, CHL, STR, SXT, SPE, TET

07-02552	Typhimurium[g]	DT12	Human	2005	Susceptible
07-02553	Typhimurium	DT12	Human	2005	Susceptible
07-02551	Typhimurium	DT120	Human	2006	AMP
07-02560	Typhimurium	DT120	Human	2006	AMP, STR, SMX, TET
07-01996	Typhimurium	DT104L	Broiler	2005	AMP, CHL, FLO, NAL, SMX, STR, SPE, TET
05-05227	Paratyphi B dT+[h]		Broiler	2005	AMP, NAL, STR, SMX, SPE, SXT, TET, TMP
06-02243	Paratyphi B dT+		Broiler	2006	AMP, NAL, SMX, SPE, SXT, TET, TMP
07-01993	Livingstone[i]		Broiler	2004	AMP, STR, SMX, SPE, SXT, TMP
07-01994	Livingstone		Broiler	2006	Susceptible
04-03524	Infantis[j]		Broiler	2004	Susceptible
07-02562	Infantis		Human	2007	Susceptible
07-02000	Enteritidis[k]	PT4	Broiler	2002	Susceptible
07-02001	Enteritidis	PT1	Broiler	1999	Susceptible

[a] PFGE, pulsed-field gel electrophoresis; *Xba*I restriction
[b] AMC, amoxicillin/clavulanic acid; AMP, ampicillin; CHL, chloramphenicol; FLO, florfenicol; GEN, gentamicin, KAN, kanamycin; NEO, neomycin; NAL, nalidixic acid; SPE, spectinomycin; STR, streptomycin; SMX, sulfamethoxazol; SXT, trimethoprim/sulfamethoxazol; TET, tetracycline; TMP, trimethoprim. Breakpoints were adapted from the Clinical and Laboratory Standard Institute (www.clsi.org), the European Committee of Antimicrobial Susceptibility testing (www.eucast.org), the Antibiotic Resistance in bacteria of animal origin I (http://cordis.europa.eu/data/PROJ_FP5/ACTIONeqDndSESSIONeq112482005919ndDOCeq169ndTBLeqEN_PROJ.htm) and the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme 2001 (http://www.danmap.org/) were used
[c] serovar Schwarzengrund antigenic formula: 4,12:d:1,7
[d] serovar Stanley antigenic formula: 4,12:d:1,2
[e] serovar Duisburg antigenic formula: 4,12:d:enz_{15}
[f] serovar Derby antigenic formula: 4,12:f,g:1,2
[g] serovar Typhimurium antigenic formula: 4,12:i:1,2
[h] serovar Paratyphi B dT+ antigenic formula: 4,12:b:1,2
[i] serovar Livingstone antigenic formula: 6,7:14:d:l,w
[j] serovar Infantis antigenic formula: 6,7,14:r:1,5
[k] serovar Enteritidis antigenic formula: 9,12:g,m:-

Table A4: *Salmonella* isolates used in this study and their characteristics.

BfR-No., Serovar	Country[b]	Source	Year of isolation	O:5[c]	Phenotypic resistance[d]	Genotypic resistance	other integron / transposon related genes
07-01645, SPB dT+[a]	NL	pig	2005	-	AMP-STR-SPE-SMX-TET-TMP-SXT	*bla*$_{\text{tem1-like}}$-[e]*[aadA1-aadA2]-sul1-tet*(A)-*dfrA1-like*	*int1, qacEΔ1, merA, int2, sat1(Tn7)*
07-01646, SPB dT+	NL	broiler	2006	-	KAN-NEO-STR-SPE-SMX-TET-TMP-SXT	*aphA1-[aadA1-aadA2]-sul1-tet*(B)-*dfrA1-like*	*int1, qacEΔ1, merA, int2, sat1(Tn7)*
07-01647, SPB dT+	NL	pig	2006	-	STR-SPE-TMP	*[aadA1-aadA2]-dfrA1-like*	*int2, sat1(Tn7)*
07-01648, SPB dT+	NL	broiler	2006	-	NAL-STR-SPE-SMX-TMP-SXT	*aadA1-sul2-dfrA1-like*	*int2, sat1(Tn7)*
07-01649, SPB dT+	NL	human	2006	-	AMP-NAL-STR-SPE-SMX-TET-TMP-SXT	*bla*$_{\text{tem1-like}}$-*aadA1-sul2-tet*(A)-*dfrA1-like*	*int2, sat1(Tn7)*
07-01650, SPB dT+	NL	pig	2006	-	NAL-STR-SPE-TMP	*[aadA1-aadA2]-dfrA1-like*	*int2, sat1(Tn7)*
07-01651, SPB dT+	NL	broiler	2006	-	AMP-NAL-STR-SMX-TET-TMP-SXT	*bla*$_{\text{tem1-like}}$-*aadA1-sul2-tet*(A)-*dfrA1-like*	*int2, sat1(Tn7)*

07-01652, SPB dT+	NL	broiler	2007	-	AMP-AMC-KAN-NEO-STR-SPE-TET-TMP	*bla*$_{\text{tem1-like}}$-*aphA1-[aadA1-aadA2-strA-strB]-*[f]*sul2-tet*(A)-*dfrA1-like*	*int1, merA, int2, sat1(Tn7)*
07-01653, SPB dT+	NL	human	2007	+	AMP-CHL-FLO-STR-SPE-SMX-TET	[g]*bla*$_{\text{pse1}}$-*floR-aadA2-sul1-tet*(G)	*int1, qacEΔ1*
07-01654, SPB dT+	NL	human	2007	-	STR-SPE-TMP	*[aadA1-aadA2]-dfrA1-like*	*int2, sat1(Tn7)*
07-01763, SPB dT+	NL	chicken	2007	-	NAL-STR-SPE-SMX-TMP-SXT	*aadA1-sul2-dfrA1-like*	*int2, sat1(Tn7)*
07-01764, SPB dT+	NL	chicken	2007	-	NAL-STR-SPE-SMX-TMP-SXT	*[aadA1-aadA2]-sul2-dfrA1-like*	*int2, sat1(Tn7)*
07-01758, SPB dT+	B	chicken	2006	-	AMP-EFT-STR-SPE-SMX-TET-TMP-SXT	[h]*bla*$_{\text{tem1-like}}$-*aadA1-sul1-tet*(A)-*dfrA1-like*	*int1, qacEΔ1, merA, int2, sat1(Tn7)*
07-01759, SPB dT+	B	chicken	2006	-	AMP-NAL-STR-SPE-TMP	*bla*$_{\text{tem1-like}}$-*aadA1-dfrA1-like*	*int2, sat1(Tn7)*
07-01760, SPB dT+	B	chicken	2006	-	AMP-NAL-STR-SPE-SMX-TMP-SXT	*bla*$_{\text{tem1-like}}$-*aadA1-sul2-dfrA1-like*	*int2, sat1(Tn7)*
07-01761,	B	chicken	2006	-	AMP-STR-SPE-SMX-	*bla*$_{\text{tem1-like}}$-*[aadA1-*	*int2, sat1(Tn7)*

SPB dT+					TMP-SXT	*aadA2]-sul2-dfrA1-like*	
07-01762, SPB dT+	B	chicken	2006	-	AMP-STR-SPE-SMX-TMP-SXT	*bla*$_{\text{tem1-like}}$*-[aadA1-strA/B]-sul1-dfrA1-like*	*int1, qacEΔ1, int2, sat1(Tn7)*
07-01765, SPB dT+	B	chicken	2007	-	AMP-NAL-STR-SPE-SMX-TMP-SXT	*bla*$_{\text{tem1-like}}$*-[aadA1-aadA2]-sul2-dfrA1-like*	*int2, sat1(Tn7)*
07-01766, SPB dT+	B	chicken	2007	-	AMP-STR-SPE-SMX-TMP-SXT	*bla*$_{\text{tem1-like}}$*-aadA1-sul2-dfrA1-like*	*int2, sat1(Tn7)*
07-01767, SPB dT+	B	chicken	2007	-	AMP-AMC-EFT-STR-SPE-SMX-TET-TMP-SXT	*[bla*$_{\text{tem1-like}}$ *-bla*$_{\text{CTX-M2}}$*]-[aadA1-aadA2]-sul1-tet*(A)*-dfrA1-like*	*int1, qacEΔ1, merA, int2, sat1(Tn7)*
07-01980, *S.* 4,5,12:b:-	UK	feed	2002	+	Susceptible	-	-
07-01981, SPB dT+	UK	chicken	2002	+	AMP-STR-SPE-SMX-TMP-SXT	*bla*$_{\text{tem1-like}}$*-aadA1-sul2-dfrA1-like*	*int2, sat1(Tn7)*
07-01982, SPB dT+	UK	feed ingredients	2003	+	Susceptible	-	-
07-01983, SPB dT+	UK	chicken	2003	-	AMP-STR-SPE-SMX-TMP-SXT	*bla*$_{\text{tem1-like}}$*-[aadA1-aadA2]-sul2-dfrA1-like*	*int2, sat1(Tn7)*
07-01984,	UK	environment	2003	+	Susceptible	-	-

S. 4,5,12:b:-

07-01985, SPB dT+	UK	bovine	2004	+	AMP-CHL-FLO-STR-SPE-SMX-TET	[g]*bla*$_{pse1}$-*floR-aadA2-[sul1-sul2]-tet*(G)	*int1, qacEΔ1*
07-01986, SPB dT+	UK	bovine	2004	+	AMP-CHL-FLO-STR-SPE-SMX-TET	[g]*bla*$_{pse1}$-*floR-aadA2-sul1-tet*(G)	*int1, qacEΔ1*
07-01987, SPB dT+	UK	feed ingredients	2005	-	AMP-STR-SPE-SMX-TET-TMP-SXT	[g]*bla*$_{tem52}$-*aadA1-sul1-tet*(A)-*dfrA1-like*	*int1, qacEΔ1, merA, int2, sat1(Tn7)*
07-01988, SPB dT+	UK	dog	2005	+	Susceptible	-	-
07-01989, SPB dT+	UK	chicken	2007	+	CHL-FLO-KAN-NEO-NAL-STR-SPE-SMX-TET-TMP-SXT	*[catA1-floR]-aphA1-[aadA1-aadA2]-sul2-tet*(A)-*dfrA1-like*	*int1, qacEΔ1, merA, int2, sat1(Tn7), sat1(int2)*
07-01990, SPB dT+	UK	feed ingredients	2007	+	Susceptible	-	-
05-05227, SPB dT+	D	chicken	2005	-	AMP-NAL-STR-SPE-SMX-TET-TMP-SXT	*bla*$_{tem1-like}$–*[aadA1-aadA2]-sul2-tet*(A)-*dfrA1-like*	*int1, int2, sat1(Tn7)*
06-01445, SPB dT+	D	chicken	2005	-	NAL-STR-SPE-TMP	*[aadA1-aadA2]-dfrA1-like*	*int2, sat1(Tn7)*

06-02243, SPB dT+	D	chicken	2006	-	AMP-NAL-STR-SPE-SMX-TET-TMP-SXT	*bla*$_{\text{tem1-like}}$*-[aadA1-aadA2]-sul2-tet*(A)*-dfrA1-like*	*int1, int2, sat1(Tn7)*
06-02522, SPB dT+	D	broiler	2006	-	NAL-STR-SPE-TMP	*[aadA1-aadA2]-dfrA1-like*	*int2, sat1(Tn7)*
06-02671, SPB dT+	D	broiler	2006	-	NAL-STR-SPE-TET-TMP	*[aadA1-aadA2]-tet*(A)*-dfrA1-like*	*int2, sat1(Tn7)*
07-04825, SPB dT+	D	pig	2007	-	AMP-AMC-EFT-NAL-STR-SPE-SMX-TET-TMP-SXT	*[bla*$_{\text{tem1-like}}$*-bla*$_{\text{CTX-M2}}$*]-[aadA1-aadA2]-sul1-tet*(A)*-dfrA1-like*	*int1, qacEΔ1, merA, int2, sat1(Tn7)*
08-00832, SPB dT+	D	spice	2008	-	AMP-AMC-EFT-NAL-STR-SPE-SMX-TMP-SXT	*[bla*$_{\text{tem1-like}}$*-bla*$_{\text{CTX-M2}}$*]-[aadA1-aadA2]-sul1-dfrA1-like*	*int1, qacEΔ1, merA, int2, sat1(Tn7)*
08-01565, *S.* 4,5,12:b:-	DK	human	2007	+	Susceptible	-	-
08-01566, *S.* 4,5,12:b:-	DK	human	2007	+	Susceptible	-	-
08-01567, *S.* 4,5,12:b:-	DK	human	2007	+	Susceptible	-	-

[a] SPB dT+ abbreviation for *S*. Paratyphi B dT+

[b] Country strain isolations NL: Netherlands, B: Belgium, UK: United Kingdom, D: Germany

[c] O:5 antigen status, when the O:5 surface antigen is present it is marked with a plus, a minus indicates the absence of this antigen

[d] AMP, ampicillin; AMC, amoxy clavulanic acid; CHL, chloramphenicol; EFT, ceftiofur; FLO, florfenicol; KAN, kanamycin; NEO, neomycin; NAL, nalidixic acid; SPE, spectinomycin; STR, streptomycin; SMX, sulfamethoxazol; SXT sulfametoxazol and trimetoprim, TET, tetracyclin; TMP, trimethoprim

[e] brackets indicate genes conferring resistance to the same antibiotic class

[f] microarray data revealed presence of *sul2* gene

[g] for these strains PCR analysis of 5' CS-3'CS integron class 1 resulted in 1200/1000 base pairs

[h] for strain 07-01758 bla_{tem52} was identified by sequencing encoding for ceftiofur resistance