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The Role of Horizontal Gene Transfer in the Evolution of Selected Foodborne Bacterial Pathogens

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Key words: *E. coli* O157:H7, *L. monocytogenes*, *Salmonella*, *S. aureus*, conjugation, transformation, transduction, mobile genetic elements

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1 *Abbreviations:* A/E, attaching and effacing; DNA, Deoxyribonucleic acid; EAST1,
2 enteroaggressive heat stable toxin; EHEC, enterhemorrhagic *E. coli*; EPEC,
3 enteropathogenic *E. coli*; GUD, β -glucuronidase; ICE, integrative conjugative
4 element; IS, insertion sequence; kb, kilobase; LAPI, Listeria pathogenicity island;
5 LEE, locus of enterocyte effacement; LRR, leucine rich repeat; PAI, pathogenicity
6 island; PVL, panton-valentine leukocidin; RNA, ribonucleic acid; rRNA, ribosomal
7 RNA; Repeats in Toxin, RTX; SAPI, *Staphylococcus aureus* pathogenicity island;
8 SCC, staphylococcal cassette chromosome; SED, *Staphylococcus* enterotoxin D; SER,
9 *Staphylococcus* enterotoxin R, SEJ, *Staphylococcus* enterotoxin J; SF, sorbitol
10 fermenting; SGI, *Salmonella* genomic island; SHEAST, sequence homologous to
11 enteroaggressive heat stable toxin; *Salmonella* outer protein E, SopE; SOR, sorbitol;
12 SPI, *Salmonella* pathogenicity island; *Salmonella* protein tyrosine phosphatase P,
13 SptP; spv, *Salmonella* plasmid virulence; ssDNA, single stranded DNA; secretion
14 system effector genes, *sse*; secretion system regulator genes, *ssr*; Stx, Shiga toxin;
15 tRNA, transfer RNA; TTSS, type three secretion system; VAP, virulence associated
16 protein;

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18

1 **Abstract**

2 Bacteria use various ways to transfer genetic information. These methods include:
3 conjugation, which requires cell to cell contact between cells, transduction, which is
4 bacteriophage-facilitated transfer of genetic information, and transformation, which is
5 the uptake of free DNA from the environment. Usually the genes to be transferred lie
6 on mobile genetic elements, pieces of DNA that encode proteins important to
7 facilitate movement of DNA within or between genomes. This review highlights the
8 transfer methods and the role of the assorted mobile genetic elements in the evolution
9 of four foodborne bacterial pathogens: *Escherichia coli* O157:H7, *Salmonella*,
10 *Staphylococcus aureus* and *Listeria monocytogenes*.

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1 **1.1 Introduction**

2 The question of “what makes a pathogen a pathogen?” has been debated over the
3 years. According to Koch’s principles, a pathogen is different to a non-pathogen due
4 to the acquisition of certain virulence traits. In other words, a pathogen differs from a
5 non-pathogen in their gene content, specifically virulence gene content (Groisman and
6 Casadesus, 2005). Casadevall et al. (1999) describe virulence as “the relative capacity
7 of a microbe to cause damage in a susceptible host”. The dissemination of virulence
8 determinants between species is of major significance affecting the evolution of
9 emergent pathogens.

10

11 The mechanisms underlying evolutionary processes and emergence of bacterial
12 pathogens are not trivial. After many generations, avirulent bacteria become virulent
13 due to several different mechanisms. Thereby, genetic material is transferred from
14 bacteria that act as donor to other bacteria (recipient). Over the past few years the
15 genome sequences of a variety of bacterial species have been determined and
16 analysed, leading to comparisons between pathogenic and non-pathogenic species,
17 which will give some important insights into the manner of bacterial evolution
18 (Fitzgerald and Musser, 2001).

19

20 In this document pertinent topics will be discussed in relation to general bacterial
21 evolution: microbial genomes and their structure; horizontal gene transfer; and mobile
22 genetic elements; followed by a more in depth discussion of evolution of four major
23 food borne bacterial pathogens, two Gram-negative: *Escherichia coli* O157:H7,
24 *Salmonella* spp, and two Gram-positive: *Staphylococcus aureus*. and *Listeria*
25 *monocytogenes*.

1 **2 Microbial genomes**

2 From complete genome sequencing data, it has been elucidated that microbial
3 genomes consist of a core genome and an accessory genome. The core genome,
4 representing the essence of a species, encodes all the housekeeping genes necessary
5 for basic cellular functions. The accessory genome, representing the diversity of a
6 species, encodes functions not strictly required for growth but that add competitive
7 advantage in certain ecological niches (Feil, 2004; Hacker and Carniel, 2001)..

8

9 The pan-genome, i.e., the core genome and the accessory genome together, describes
10 the total gene repertoire of a bacterial species. Species can have either an open or
11 closed pan-genome. With an open pan-genome, organisms can live in many different
12 environments and have many opportunities for genetic exchange between different
13 species. *Esherichia coli* and *Salmonella* are examples of organisms with an open pan-
14 genome. Organisms with a closed pan-genome usually have more conserved genomes
15 and live in isolated niches with limited access to the global microbial gene pool.
16 Organisms with a closed pan-genome include *Bacillus anthracis* and *Mycobacterium*
17 *tuberculosis* (Medini et al., 2005).

18

19 The majority of genes in the core gene pool display consistent G+C content and codon
20 usage, whereas the genes in the accessory gene pool have features that are
21 characteristic of elements transferred from an external source: different G+C content,
22 codon usage and presence of mobility genes (Hacker and Carniel, 2001; Hacker et al.,
23 2004).

1 **3 Horizontal gene transfer**

2 Horizontal (or lateral) gene transfer is the transfer of genetic information between
3 bacterial cells and is different to the transfer of DNA from mother to daughter cell. It
4 is an integral factor in the generation of genetic variability and evolution in bacteria,
5 enabling species to quickly adapt to environmental variations (Burrus and Waldor,
6 2004). When compared to evolution in multicellular eukaryotes, prokaryotic evolution
7 occurs in “quantum leaps” as a result of the mechanisms and mobile genetic elements
8 involved in horizontal gene transfer (Hacker and Carniel, 2001). The massive amount
9 of bacterial genomes being sequenced to date has brought about a new appreciation
10 for and supports the concept of genome plasticity due to horizontal gene transfer
11 (Sorensen et al., 2005). Prerequisites for natural horizontal gene transfer to be
12 successful are: the need for stable integration of the gene into the genome, no
13 disturbance of regulatory or genetic structures, expression and successive production
14 of a functional protein (Susanna et al., 2006).

15

16 There are two approaches to determine horizontal gene transfer in a genome:
17 phylogenetic comparison, where different organisms are compared and the similarity
18 or dissimilarity is recognized; and parametric comparison where genes that appear
19 atypical in their current genomic context are inferred to have been introduced from a
20 foreign source. The best way of determining whether horizontal gene transfer events
21 have occurred in a genome is to apply a mixture of the two approaches. (Lawrence
22 and Ochman, 2002).

23

24 Horizontal gene transfer is a major determinant in the evolution of pathogenic
25 bacteria. Dissemination of virulence genes via this method enables non-virulent

1 strains to become virulent. All the bacteria addressed in this study have in some way
2 or another succumbed to horizontal gene transfer in order to evolve into the
3 pathogenic variant.

4
5 Prokaryotes typically obtain new DNA sequences by three methods which play a part
6 in horizontal gene transfer events in bacteria: conjugation, transduction and
7 transformation (Burrus and Waldor, 2004; Frost et al., 2005; Hacker and Carniel,
8 2001).

9 ***3.1 Conjugation***

10 Conjugative transfer is mediated by certain plasmids or integrative conjugative
11 elements with specific transfer genes. Conjugation requires cell-to-cell contact and
12 particular gene products which mediate the transfer process and the creation of the
13 conjugation pore. Many plasmids are not self-transmissible, but can be mobilized
14 from one bacterium to another in the presence of a separate self-transmissible
15 plasmid, by the use of mobilization proteins (Thomas and Nielsen, 2005).

16

17 ***3.2 Transduction***

18 Transduction is a bacteriophage-facilitated transfer of genetic material from one
19 bacterial host to another. It is a specific horizontal gene transfer event as
20 bacteriophages have a limited range of hosts (Sorensen et al., 2005). Bacteriophages
21 are specialized viruses that infect bacteria. Two types of bacteriophages exist
22 depending on their life cycle. Lytic: which are only capable of infecting bacterial cells
23 and reproducing more bacteriophage with the destruction of the bacterial host cell;
24 and lysogenic, also known as temperate bacteriophage: are able to integrate into the

1 host genome and become part of the genetic make up of the host bacterium. Under
2 these conditions the bacteriophage is known as a prophage. The lytic cycle can be
3 induced in prophages under certain environmental conditions like the antibiotic-
4 induced SOS response, a post-replication DNA repair system (Beaber et al., 2004;
5 Goerke et al., 2006). Lysogenic conversion occurs when the transferred genes confers
6 immunity to the recipient bacterium to further infection by the same or similar phage,
7 and sometimes alters the phenotype of the recipient by carrying additional genes such
8 as virulence genes. The importance of the spread of virulence genes by transduction
9 has only become evident in recent years. It has been shown that the genes encoding
10 shiga toxins, *stx1* and *stx2* are transferable from one strain of *E. coli* to another by
11 bacteriophages (Brabban et al., 2005).

12 ***3.3 Transformation***

13 Transformation is the stable uptake, integration and expression of extracellular DNA
14 that occurs in nature when bacterial species are in a physiological state known as
15 competence (Thomas and Nielsen, 2005). Natural transformation has been shown in
16 many archaea and subdivisions of bacteria. Release and persistence of extracellular
17 DNA, presence of competent cells and the ability of the DNA to be integrated into the
18 host chromosome are the requirements for natural transformation to occur.

19

20 **4. Mobile genetic elements**

21 Mobile genetic elements, segments of DNA encoding proteins important for the
22 mediation of movement of DNA within genomes, play an integral part in the
23 evolution of bacterial genomes (Frost et al., 2005), and are the backbone of horizontal
24 gene transfer.. Mobile genetic elements include plasmids, bacteriophages, genomic

1 islands, integrons, transposons and insertion sequence (IS) elements (Dobrindt et al.,
2 2004). Each element shall be discussed in the following section.

3 **4.1 Plasmids**

4 Plasmids are usually circular, self replicating DNA molecules existing in cells as
5 extrachromosomal replicons. Plasmids are found in bacteria from different
6 communities ranging from soil, marine and clinical environments and they signify a
7 distinct genetic resource as they may confer many advantageous traits such as
8 antibiotic resistance and ability to use various carbon sources (Sorensen et al., 2005).
9 Plasmids can be self-transmissible (or conjugative), meaning that they encode all the
10 necessary proteins for mobilization or they can be non self-transmissible (or non-
11 conjugative), where the mobilization proteins have to be provided *in trans*. The
12 horizontal transfer of non-conjugative plasmids has been shown in biofilms of *E. coli*
13 by Maeda et al. (2006), which may prove the importance of colony-biofilm formation
14 for plasmid transfer, and dissemination of plasmids in natural environments.

15 **4.2 Bacteriophages**

16 Bacteriophages act as facilitators of two types of horizontal genetic exchange.
17 Generalized transduction, where any gene within a donor can be transferred to a
18 recipient strain by a lytic or temperate bacteriophage and defective phage particles
19 deliver bacterial DNA into recipient bacterium cytoplasm where it either gets
20 degraded or becomes recombined into recipient DNA. Specialized transduction which
21 is a more efficient process that specifically involves transition from prophage to lytic
22 cycle. Many bacteriophages incorporate their genome into a specific point in the host
23 genome and upon induction the integrated prophage usually excises itself precisely as
24 a whole unit. Sometimes, however, this excision is imprecise and part of the host

1 genome becomes excised also, enabling the resultant daughter bacteriophage to spread
2 genetic material to new bacterial hosts. Lysogenic conversion is similar to specialized
3 transduction in that temperate bacteriophage pass through the lysogenic stage but it
4 does not involve incorrect excision of the prophage DNA. It normally confers
5 immunity to the host bacterium to further infection by the same or related
6 bacteriophage, and if the bacteriophage contains additional genes known as prophage
7 morons, that are part of the phage genome that do not have a phage function but adds
8 to the fitness of the bacterium and can alter the phenotype of the infected host
9 (Brabban et al., 2005). Many bacteria have prophages as part of their genomes, and in
10 a lot of cases certain virulence determinants actually lie on prophages (Abedon and Le
11 Jeune, 2005). Canchaya et al. (2004) studied the impact of prophages on bacterial
12 chromosomes and found that prophages are a prominent part of pathogenic bacterial
13 genomes, often containing virulence genes and adding to the genetic diversity of the
14 strains. Therefore, phages have an important role in the diffusion of virulence
15 determinants and the evolution of many bacterial pathogens (Brussow et al., 2004).

16 ***4.3 Genomic islands***

17 The concept of genomic islands was first conceived in pathogenic bacteria with the
18 discovery of particular regions of bacterial chromosomes that contribute to
19 pathogenicity (Hacker and Kaper, 2000). These regions were characterized by a
20 significant difference in G+C content, alternative codon preference, mobility genes,
21 virulence genes, and they were coined “pathogenicity islands” (PAI). Later it was
22 found that other non-pathogenic species contained similar genome regions that
23 contributed somewhat to the fitness of the organism, and the general term “genomic
24 islands” was created. These genomic islands form part of the flexible gene pool and
25 these elements were previously transferred by horizontal gene transfer as they contain

1 remnants of mobile-enabling sequences such as integrases, transposases and flanking
2 direct repeats. The majority of these genetic clusters carry functions that are useful for
3 the survival of the organism, and they provide a selective advantage over the non-
4 island carrying populations (Hacker and Carniel, 2001). There are many types of
5 genomic islands: symbiosis, fitness, metabolic, resistance and the one most relevant to
6 pathogens, pathogenicity islands (Dobrindt et al., 2004). The type of island that an
7 organism carries depends on the genetic background of the host and the ecological
8 niche in which it presents itself.

9

10 Pathogenicity islands contain one or more virulence genes, are present in the genomes
11 of pathogenic bacteria but are absent from the non-pathogenic variant of the same
12 species and often exist in the size range of 10-200 kb (Schmidt and Hensel, 2004).
13 Not much is known about the origin of these genomic islands, but it is thought that
14 they originated from integrating plasmids or phages that have lost the ability to self-
15 transfer. Genomic and pathogenicity islands have been selected during evolution as
16 they confer selective advantages to their bacterial host. Pathogens that do not contain
17 pathogenicity islands such as *Chlamydia* spp. and *Mycobacterium* spp., examples of
18 organisms with closed pan-genomes (Medini et al., 2005), exhibit an intense
19 adaptation to a specific host environment accompanied by reduction in the genome
20 size and an inability to replicate outside the host.. From this it can be inferred that the
21 presence of a pathogenicity island increases the range of habitats that can be occupied
22 by a particular bacterial species (Schmidt and Hensel, 2004).

23 **4.4 Integrons**

24 Integrons are natural cloning and gene expression mechanisms, capable of capturing
25 gene cassettes that lack promoters, using site specific recombination (Rowe-

1 Magnus.and Dazel, 2001) and are composed of integrase genes, gene cassettes and an
2 integration site for gene cassettes. They have been found on plasmids, transposons
3 and the bacterial chromosome (Fluit and Schmitz, 2004) and also provide promoters
4 for the expression of cassette genes (Hall and Collis, 1995). Flanked by inverted
5 repeats, they have the capacity to move within or between replicons. There are two
6 discrete groups of integrons: resistance integrons and super-integrons. Resistance
7 integrons carry gene cassettes that encode antibiotic or disinfectant resistant
8 determinants; super-integrons encode a variety of different functions (Fluit and
9 Schmitz, 2004). Five classes of resistant integrons (RIs) have been historically defined
10 on the basis of the divergence among their integrase genes, and each class appears to
11 be able to acquire the same gene cassettes (Mazel, 2006).

12 ***4.5 Transposons***

13 Transposons are pieces of DNA considered as “jumping genes” as they have the
14 ability to change their genetic location. They can be present on chromosomes and
15 plasmids and they encode a transposase gene, whose product, the transposase, is
16 responsible for the movement of the transposon. Transposons can carry genes for
17 antibiotic resistance as well as other properties. Inverted repeat DNA sequences flank
18 the transposons, and they have the ability to move within or between replicons.
19 Transposition events do not require homology between the target site and the
20 transposon. Conjugative transposons are genetic elements able to promote their own
21 intracellular transposition and intercellular conjugal transfer (Beuzon et al., 2004).

22

1 **4.6 IS elements**

2 Insertion sequence (IS) elements are mobile genetic elements widely distributed
3 among bacteria which can be found on both plasmids and chromosomes. Their
4 activities cause mutations which promotes genetic diversity and sometimes adaptation
5 to a new environment. IS elements are also widely considered as important for
6 horizontal gene transfer, including especially the movement of acquired genes from
7 extrachromosomal elements into the chromosome (Schneider et al., 2002). IS
8 elements usually encode a transposase gene, are flanked by inverted repeats and
9 mediate recombination by transposition events and by homologous recombination
10 between many copies of the same IS element in one genome (Mahillon and Chandler,
11 1998).

12

13 **5. *Escherichia coli* O157:H7**

14 The *Escherichia coli* O157:H7 virulence factor encoding genes are located on several
15 genetic elements. The genes of Shiga toxins (*stx1* and *stx2*) are located on lambdoid
16 bacteriophages, while the genes involved in the formation of the attaching and
17 effacing lesion are located on 35.6-kb chromosomal Locus of enterocyte effacement
18 (LEE). In addition to the LEE and Stx-converting phages, the pathogenesis of
19 enterohemorrhagic *E. coli* (EHEC) O157 infections likely involves virulence factors
20 encoded by other PAIs, other phages and by a 92 kb plasmid referred to as pO157.
21 This plasmid carries the genes governing the production of enterohemolysin and other
22 putative virulence factors, such as a catalaseperoxidase and a serine protease.

23 Whittam et al. (1993) recognized that *E. coli* O157:H7 strains are most closely related
24 genetically to enteropathogenic *E. coli* O55:H7 strains, a recent ancestor of a

1 nontoxigenic pathogenic clone associated with diarrhea occurring in infants. Feng et
2 al. (1998) generated with the help of a multilocus enzyme electrophoresis an
3 evolutionary model to predict the genetic relatedness of several Shiga toxin producing
4 *E. coli* (STEC) O157 strains. The analysis showed that STEC O157 strains
5 comprehend a cluster of five closely related electrophoretic types (ET). The
6 emergence of O157:H7 was associated with a series of steps from a nontoxigenic
7 progenitor and indicates that the most recent common ancestor (A1) of both strains
8 (*E. coli* O157:H7, *E. coli* O55:H7) harboured the pathogenicity island LEE, the uidA -
9 10 mutation (A to T) and had the abilities to ferment sorbitol (SOR+) and express β -
10 glucuronidase (GUD+). Table 1 illustrates a proposed model for the evolution of *E.*
11 *coli* O157:H7 (Feng et al., 1998). In the emergence of *E. coli* O157:H7. the next step
12 was that A1 acquired the phage Stx2 presumably by transduction, resulting in a Stx2-
13 positive O55:H7 (A2). In the step from A2 to A3, the large virulence EHEC plasmid
14 (pO157) (Levine et al., 1987) and the *rfb* region encoding O157 antigen was acquired,
15 the uidA +92 (T to G) conversion took place and the somatic antigen changed from
16 O55 to O157. Furthermore, the model predicts that from A3 two distinct lines are
17 evolved, with A3 on one branch due to a mutation in the flagellar operon that caused a
18 loss in motility (Monday et al., 2004). This ancestor (A4) retains Stx2 and is still able
19 to express β -glucuronidase as well as ferment sorbitol and in addition accumulated
20 two enzyme allele mutations resulting in the German *E. coli* O157:H⁻. In contrast in
21 the other branch, the lineage is no longer able to ferment sorbitol and acquired the
22 phage Stx1 presumably by phage conversion resulting in clone A5. Accordingly,
23 because of the lost the ability to express β -glucuronidase (Monday et al., 2001) *E.*
24 *coli* O157:H7 with SOR- and GUD- phenotypes (A6) emerged, which represent the
25 immediate ancestor of the common O157:H7 clone (Whittam et al., 1998, Feng et al.,

1 1998), a serious foodborne pathogen causing diarrhoea, hemorrhagic colitis (HC), and
2 hemolytic-uremic syndrome (HUS) which can be life threatening (Adu-Bobie, 1998,
3 Kim et al., 2001). The role of mobile genetic elements in the evolution and transfer of
4 virulence genes in *E. coli* O157:H7 will be discussed further below.

5 ***5.1 Role of bacteriophages***

6 Shiga toxin-encoding phages present highly mobile genetic elements that are involved
7 in regulation and horizontal transfer of *stx* genes (Herold, et al., 2004). The Shiga
8 toxins are the main virulence factors in the disease progression of haemorrhagic
9 colitis and haemolytic uraemic syndrome caused by Stx producing *E. coli* O157:H7.
10 Genome sequence analyses showed that a higher percentage (nearly 85%) of the
11 variably present genes e.g. *vap* is bacteriophage related and indicates that phages play
12 a dominant role in diversification of the chromosomal architecture of *E. coli* O157
13 strains (Hayes et al., 1995; Perna et al., 2001) as well as a in the evolution of O157:H7
14 (Hayashi et al., 2001). Transduction of *stx* genes by phages to other enteric *E. coli*
15 strains could contribute to the detected heterogeneity in STEC serotypes as have been
16 recovered more than 400 different O:H serotypes of STEC from animals, foods
17 (Johnson et al., 1996) and from human infection (Blanko et al., 2004).

18 The acquisition of phage-borne toxin genes was probably early and some genes were
19 conserved, but there is evidence of dynamic turnover in phage genes which results
20 from phage replacement, localized recombination and island erosion (Ohnishi et al.,
21 2002; Shaikh and Tarr, 2003; Wick et al., 2005). Results of the comparative analysis
22 of the genome suggest that prophages carried out a variation and gained the additional
23 genes (or the prophages were replaced or recombined with other phages) to achieve
24 the gene complement of *E. coli* O157 Sakai, which has the Stx1 phage (or truncated

1 Stx1 phage occupying *yehV* (integration sites) and Stx2 phage occupying *wrbA*
2 (integration sites) (Wick et al., 2005).

3

4 In several studies transduction of *stx* genes has been shown *in vitro* (Schmidt et al.,
5 1999; Scotland et al., 1983; Strauch et al., 2001) and *in vivo* (Acheson et al., 1998;
6 Toth et al., 2003). Schmidt et al. (1999) showed using a model of a detoxified
7 derivative of a Stx2-converting bacteriophage that Stx2-converting bacteriophages
8 have the ability to spread among enteric *E. coli* strain. These findings support the
9 important role for Stx-encoding bacteriophages in the spread of *stx* genes among
10 enteric *E. coli* strains.

11 **5.2 Role of plasmids**

12 *E. coli* O157:H7 contains plasmids, which generally carry genes harbouring virulence
13 factors and represent extra chromosomal elements.. There are three known plasmids
14 associated with *E. coli* O157:H7. A large plasmid of approximately 92 kb (pO157),
15 called EHEC plasmid (Karch et al. 1998), present in virtually all clinical isolates. The
16 other two plasmids are small, 6.7 and 3.3 kb in size, and are present less frequently
17 (Levine et al., 1987; Ostroff et al., 1989; Paros et al., 1993). Large plasmids of STEC
18 most likely have been acquired by horizontal DNA transfer, as they are encoded by
19 accessory genetic elements (Pradel et al., 2001).

20

21 The haemolysin gene EHEC-*hlyA* harboured on pO157 is present in sorbitol-
22 fermenting (SF) *E. coli* O157 and non-sorbitol-fermenting (NSF) *E. coli* O157:H7,
23 but the plasmids of SF *E. coli* O157:H- strains lack *espP*, encoding an extracellular
24 serine protease, and *katP*, encoding a catalase/peroxidase,. NSF *E. coli* O157:H7 and
25 SF *E. coli* O157 may have acquired different plasmids after their evolutionary split

1 from a common ancestral EPEC-like O55:H7 into two lineages..There is also the
2 possibility that one common plasmid, harboured by the ancestor and carrying the
3 *hlyA*, *espP* and *katP* genes, lost the *espP* and *katP* markers in the SF *E. coli* O157:H-
4 clone. Another presumption of Brunder et al. (1999) could be that the ancestral
5 plasmid has gained the two markers only in the NSF *E. coli* O157:H7 lineage, maybe
6 enhancing the survival and spread of this clone to survive and spread. The presence of
7 mobilization genes on plasmids renders them capable of horizontal transfer, so these
8 plasmids should be self transmissible or mobilizable. The plasmid (pO157) of *E. coli*
9 O157:H7 is reported to be transfer-deficient (Hales et al., 1992; Makino et al., 1998)
10 but it is presumed that it was transferable during some earlier phase of evolution.
11 The EspP homologues of other *E. coli* pathogroups share only about 60% sequence
12 identity at a protein level (Brunder et al., 1997) and suggests that no exchange of these
13 genes between *E. coli* pathogroups occurs at present. This transfer barrier may be
14 caused by differences in habitats of the groups or incompatibility of plasmids
15 occurring in the various groups (Brunder et al., 1999).

16

17 ***5.3 Role of integrons***

18 Integrons are DNA elements that may contain transferable antimicrobial resistance
19 gene cassettes. Until now, five classes of integrons have been identified with class 1
20 being the most widespread (Singh et al., 2005). The study of Zhao et al. (2001) reports
21 the presence of class 1 integrons conferring multiple resistance phenotypes among
22 EHEC strains isolated from cattle, ground beef, and humans, which is presumable the
23 first report on the presence of integrons in O157:H7 and non-O157 EHEC strains.
24 Class 1 integrons located on mobile plasmids have facilitated the emergence and

1 dissemination of antimicrobial resistance among STEC in humans and food animals
2 (Singh et al., 2005).

3

4 ***5.4 Role of insertion sequences***

5 O-islands in O157:H7 (genomic regions specific to *E. coli* O157:H7 or phage regions
6 of *E. coli* O157:H7) _correspond to many of the insertion sites of IS elements in *E.*
7 *coli* B and K-12. This association indicates that these regions of the chromosome
8 underwent chromosomal rearrangements, in particular either the insertion or deletion
9 of the DNA regions that distinguish the different strains; in the case of insertions, the
10 large-scale differences also imply horizontal transfer. Insertion sequence elements are
11 well known to play important roles in gene deletion events and in the incorporation of
12 foreign DNA into the chromosome during and following horizontal gene transfer.
13 Data of Schneider et al. (2002) reveal a high level of IS activity since *E. coli* B, K-12,
14 and O157:H7 diverged from a common ancestor, including IS association with
15 deletions and incorporation of horizontally acquired genes as well as transpositions.

16

17 The study of Hsu and Chen (2003) showed that the differences in the sequences and
18 the transposition activities of IS1 elements indicated that they had been transferred
19 from *S. boydii* into *E. coli* O157:H7, and then into *E. coli* K12. Horizontal transfer
20 and vertical inactivation, therefore, were suggested as two mechanisms in the
21 domestication of these IS1 elements in *S. boydii* and *E. coli* (Hsu and Chen, 2003).
22 These findings demonstrate the importance of IS elements in genome plasticity and
23 divergence.

24

1 The enteroaggregative *E. coli* heat-stable enterotoxin 1 (EAST1) represents a
2 virulence factor whose significance in the pathogenicity is not well characterised to
3 date, and shows a wide distribution among diarrheagenic *E. coli* (Karch et al., 1999).
4 Yamamoto et al. (2000) investigated the EAST1 gene homologues of EHEC strains
5 from outbreaks as well as sporadic cases in Japan. They found sequence homologous
6 to the EAST1 gene in *Y. pestis*. The location of it was within an IS sequence (IS285).
7 A comparison of sequence demonstrated that type 1 SHEAST (sequence homologous
8 to the EAST) is closer to the EAST1 gene (89.7% similarity in sequence) than to the
9 EAST1-like sequence of IS285 (76.9% homology in sequence). Theoretically the
10 EAST1 gene, type 1 SHEAST, and the EAST1-like sequence of IS285 are changeable
11 to each other by single base substitutions. This suggests that they have originated
12 from a sequence from a common ancestor. The results of the study indicated cross-
13 species transfer of the EAST1 gene sequences between *E. coli* and *Y. pestis*. The
14 EAST1 gene sequences may have derived from part of the genes involved in
15 transposition events (Yamamoto et al., 2000).

16

17 ***5.5 Role of pathogenicity islands***

18 Attaching and effacing lesions present one of the most important features of EHEC
19 O157 (Kaper et al., 1998) and are located on a large chromosomal pathogenicity
20 island (PAI) termed as LEE (Locus of Enterocyte Effacement). It is thought that LEE
21 harbours not only the genes for the secretion and translocation apparatus, but also the
22 genes of effectors that may be secreted through the system.

23

24 No clear evidence exists for the spread of LEE in nature. However, LEE has a 38.4%
25 G-C content (Elliot et al. 1998), in comparison to 50.8% for the *E. coli* chromosome

1 (Blattner et al. 1997), and the occurrence at different genomic locations in divergent
2 clonal groups (Wieler et al. 1997) demonstrates that LEE has been horizontally
3 transferred in the past (McGraw, 1999). It is reported by Elliott et al. (1998) that the
4 genetic organisation of the LEE from EHEC O157:H7 is similar to that reported for
5 LEE from enteropathogenic *E. coli* (EPEC) O127:H6. That the LEE has different
6 locations in different EHEC phylogenetic lineages suggest the insertion of LEE was at
7 multiple times and sites during the evolution of EHEC and EPEC (Boerlin et al. 1998;
8 Wieler et al.; 1997; Kaper et al.; 1998; Rumer et al.; 2003). Finally, several studies
9 (e.g. Morabito et al., 2003; Shen et al., 2004; Tauschek et al., 2002) support the fact
10 for considerable plasticity in *E. coli* O157:H7 strain EDL933 genomic islands, which
11 may play a role in the contribution to the on-going evolution of EHEC (Shen *et al.*,
12 2004).

13

14 **5.6 Role of vesicles**

15 A vesicle is a relatively small and enclosed compartment, derived from membranes.
16 Vesicles may contain lipopolysaccharide, periplasmic proteins, outer membrane
17 proteins, phospholipids, hydrolytic enzymes, DNA, RNA and other elements
18 associated with the virulence of the producing bacteria (Dorward et al., 1989;
19 Kadurugamuwa et al., 1995) As well as genetic elements, vesicles may play a role in
20 the transfer of genetic material among similar bacterial species (Deich and Hoyer,
21 1982; Dorward *et al.*, 1989; Kolling and Matthews, 1999; Yaron et al., 2000). *E. coli*
22 O157:H7, like other species such as *Salmonella* spp or *Campylobacter jejuni*, is able
23 to produce membrane vesicles which may play a role in virulence (Kolling et al.,
24 1997; Wai et al., 1995). A study showed that isolation of membrane vesicles from *E.*
25 *coli* O157:H7 facilitated transfer of genes to *Salmonella enterica* serovar Enteritidis or

1 *E. coli* JM109, and these genes were subsequently expressed by recipients (Yaron et
2 al., 2000). The integration of virulence genes is possibly a regulated event, thereby
3 allowing the transfer of specific genes to other enteric bacteria, thus facilitating
4 genetic divergence (Kolling et al., 1997). However, whether vesicles played a role in
5 the evolution of *E. coli* O157:H7 or EHEC has not been established.

6 **5.7 Conclusion**

7 Within serogroup O157 many of the putative virulence factors are harboured on
8 mobile genetic elements, such as phages, plasmids and pathogenicity islands, which
9 have been subject to numerous insertions and deletions (Boerlin et al., 1998). The
10 majority of EHEC virulence factors have been acquired by horizontal transfer of
11 genetic material. Thereby the acquisition of the LEE that encoded the genes involved
12 in the formation of the attaching and effacing (A/E) lesion and the acquisition of the
13 phage-encoded Stx genes present two crucial steps in the evolution of EHEC O157
14 from a commensal ancestor. Phages could be the major contributors in the evolution
15 of EHEC virulence and in the emergence of new EHEC clones (Caprioli et al., 2005;
16 Hayashi et al., 2001).

17 **6. Salmonella**

18 The genome of *Salmonella* consists of a variety of mobile genetic elements that have
19 played a role in the evolution of this pathogen. Many virulence determinants of this
20 organism reside on a veritable assortment of prophages, integrons, pathogenicity
21 islands and plasmids and the evolution of *Salmonella* into a pathogen has been
22 marked by the acquisition of these elements which contribute to the virulence
23 capability of this organism (Groisman and Ochman, 1997). Examples of these
24 virulence factors include the *pef* fimbrial operon, which lies on the virulence plasmid,

1 the genes encoding the type three secretion system- 1 (TTSS-1) (Marcus et al., 2000),
2 and the genes encoding lipopolysaccharide (LPS) which is encoded on the
3 chromosome (Fierer and Guiney, 2001). Analysis of the completed genome sequence
4 of *S. Typhimurium* LT2 has lead to the conclusion that many gene blocks have been
5 acquired by horizontal gene transfer since the divergence of this organism from *E.*
6 *coli* around 100 million years ago (Porwollik and McClelland, 2003).

7 **6.1 Role of bacteriophages**

8 A wide array of phage and phage remnant genes make up the *Salmonella* genome,
9 which have a huge effect on the ability for lateral gene transfer events and the
10 dissemination of virulence factors in this organism. Lysogenic conversion, the process
11 of altering the host phenotype that occurs upon the integration of the virulence factor-
12 encoding prophages into the host chromosome, has been a huge influence on the
13 evolution of the virulent phenotype of *Salmonella*. The bacteriophage-encoded
14 proteins which are involved in lysogenic conversion provide mechanisms for
15 *Salmonella* to invade tissues, avoid immune defences and to damage cells of the host
16 (Boyd and Brussow, 2002). Most of the temperate phages associated with *Salmonella*
17 belong to the P22 family that are capable of facilitating horizontal gene transfer by
18 transduction (Schicklmaier et al., 1998). Some *Salmonella* serovars can contain up to
19 seven prophages on their genome, many which are cryptic with unproven activity as
20 yet. *S. Typhimurium* LT2 has four functional phage genomes: P2-like Fels-2, and λ -
21 like Fels-1, Gifsy-1, and Gifsy-2. These prophages respond to DNA stress by excision
22 and replication (Porwollik and McClelland, 2003). In some cases the excision of a
23 prophage, like Gifsy-2 from the genome of *S. Typhimurium*, results in the attenuation
24 of systemic infection. Transferability of genes encoded on phages within different
25 *Salmonella* serovars was originally just thought to occur between phages of the same

1 family, but Miold et al. (2001) demonstrated that genes could be transferred across
2 phages from different families when effector genes were revealed to be encoded by a
3 P2-like phage in *S. Typhimurium* and Typhi, and in a cryptic λ -like phage in *S. Hadar*,
4 *S. Gallinarum*, *S. Enteritidis* and *S. Dublin*. An obstacle for the horizontal gene
5 transfer of virulence genes via phage is the immunity to superinfection that the
6 resident prophages impose on the bacterial strain. This problem is evaded by the
7 ability of unrelated phages to transfer virulence factors. This may explain why
8 *Salmonella* spp. are able to adapt rapidly to a broad range of host vectors. Two
9 generalized transducing phages, ES18 and PDT17, have been used to transfer
10 resistance elements of *Salmonella* Typhimurium DT104 by Schmieger and
11 Schicklmaier (1999), with PDT17 being a prophage of *S. Typhimurium* strains.
12 PDT17 is therefore capable of spreading resistance genes to other organisms.

13

14 The SOS response is induced by antibiotics such as trimethoprim, which inhibits
15 DNA replication and ciprofloxacin which exposes ssDNA. The “damaged” bacterial
16 DNA interacts with and activates the multifunctional protein RecA, which promotes
17 the autoproteolysis of repressor of DNA functions, LexA and phage repressor CI. The
18 decline in CI causes derepression of the phage lytic genes resulting in the resumption
19 of lytic growth (Goerke et al., 2006). In general it has been shown that phages play a
20 very important role in the dissemination of virulence determinants in *Salmonella*
21 (Figueroa-Bossi et al., 2001).

22

23 **6.2 Role of integrons**

24 The spread of antibiotic resistance in *Salmonella* has mainly been attributed to
25 integrons. Five classes of resistance integrons are described (Mazel, 2006), but only

1 Class I and Class II are found in *Salmonella* (Fluit, 2005). Multidrug resistance
2 elements in *Salmonella* reside on a *Salmonella* Genomic Island (SGI) region 1, which
3 is a 43 kb region that contains a 13 kb gene cluster that confers resistance to a variety
4 of antibiotics. This region is made up of a complex class 1 integron that belongs to the
5 In4 group (Doublet et al., 2005). This SGI1 region was transferred by conjugation
6 from different serovars of *S. enterica* to other *S. enterica* and *E. coli* strains, with the
7 help of helper plasmid R55. In the absence of plasmid R55, no transfer of the SGI1
8 region occurred proving that this element is non-self-transmissible but is mobilizable,
9 and this mobility contributes to the spread of antibiotic resistance among serovars of
10 *S. enterica*. In Portuguese study by Antunes et al. (2005) it was shown that the
11 majority of sulphonamide resistance genes were carried on class I integrons (Antunes
12 et al., 2005).

13

14 **6.3 Role of plasmids**

15 Many enteropathogenic bacteria require plasmid-encoded factors for full virulence
16 expression, *Salmonella* included (Porwollik and McClelland, 2003). Most *Salmonella*
17 serovars contain a virulence plasmid ranging from 50-90kb in size, which is necessary
18 for the multiplication of bacteria in the reticulo-endothelial system of warm-blooded
19 vertebrates. Only a 7.8 kb *spv* region (*Salmonella* plasmid virulence) seems to be
20 necessary to confer the virulence phenotype (Rotger and Casadesus, 1999).. Virulence
21 plasmids from some of the serovars are self-transmissible, supporting the possibility
22 of conjugative spread of this plasmid. *S. Typhi* CT18 contains two plasmids, pHCM1,
23 a 281 kb plasmid which confers antibiotic resistance and, pHCM2, a 107 kb plasmid
24 which displays high sequence similarity to the virulence associated plasmid of *Y.*

1 *pestis*, pointing to the possibility that lateral gene transfer occurred between these
2 distantly related genera or that they had a common ancestor (Porwollik and
3 McClelland, 2003). Plasmids have shown to have a role in the transfer of genes in
4 *Salmonella* in a report by Ferguson et al. (2002), where they showed that antibiotic
5 resistance plasmids can be transferred from plasmid-containing strains of *S.*
6 Typhimurium to plasmid-free strains of *S.* Typhimurium intracellularly in human
7 epithelial cells by conjugation. Multidrug resistant plasmids from *S.* Typhimurium
8 were shown to be transferred to *E. coli* by conjugation (Gebreyes et al., 2002),
9 showing the ability for these plasmids to be disseminated into different species.

10

11 ***6.4 Role of insertion sequences***

12 Many *Salmonella* genomes carry a range of IS elements which are tightly associated
13 with genomic rearrangements and duplications in *Salmonella*. Each *Salmonella*
14 genome may contain between 30 and 75 transposase genes, introduced by phage,
15 transposon or IS elements, which may promote the movement of certain genes around
16 the genome (Porwollik and McClelland, 2003).

17

18 ***6.5 Role of pathogenicity islands***

19 *Salmonella* contains a number of pathogenicity islands, regions of the genome
20 characterized by a number of properties: their absence from non-pathogenic strains,
21 their differing G+C content and by their differing codon usage from the rest of the
22 *Salmonella* genome. To date there are 10 recognised *Salmonella* pathogenicity islands
23 (SPIs) (Bishop et al., 2005) and they are outlined in table 2. These agents are thought

1 to have been acquired by horizontal gene transfer due to the presence of agents of
2 DNA mobility (integrases, transposases, direct repeats) (Hensel, 2004).

3

4 The best characterised *Salmonella* Pathogenicity Island (SPI), SPI-1 , encodes the
5 component genes for a type III secretion system (TTSS), as well as a plethora of
6 regulators and effectors. This TTSS is responsible for the translocation of effectors
7 encoded by SPI-1 and also for some additional genes encoded outside SPI-1. Overall,
8 SPI-1 is a region of about 40kb, encoding around 29 genes (Marcus et al., 2000). SPI-
9 1 was found to be present in all the *S. bongori* and *S. enterica* subspecies that have
10 been analyzed to date (Hensel, 2004). Invasion of *Salmonella* is mediated by delivery
11 of effectors such as SopE (*Salmonella* outer protein E), SopB (with a loci on SPI-5)
12 SptP (*Salmonella* protein tyrosine phosphatase P) that directly engage host cell
13 signalling pathways, modifying the cytoskeleton, allowing uptake of the bacterial cell.
14 TTSS from SPI-1 form needle-like appendages that facilitate the delivery of the
15 effector proteins. SPI-1 functions are needed for the invasion of non-phagocytic cells
16 (Schmidt and Hensel, 2004). The *Sip*ABCD genes are involved in the invasion of
17 cultured epithelial cells, with *SipA* binding directly to actin in the host cell, inhibiting
18 their depolymerization. *SipB* and *SipC* can act as effectors and as translocators for
19 other effector proteins of SPI-1 (Marcus, Brumell, et al. 2000). Genes that are
20 associated with DNA mobility are absent from SPI-1 (Hensel, 2004), and this locus
21 seems to be stable in clinical *Salmonella* strains analyzed thus far (Schmidt and
22 Hensel, 2004).

23

24 *Salmonella* Pathogenicity Island 2, SPI-2, encodes a TTSS and a two component
25 regulatory system. This TTSS (TTSS-2) is structurally and functionally distinct from

1 the TTSS from SPI-1 (Marcus et al., 2000) and is activated when the bacteria are
2 intracellular. Comparison of SPI-1 and SPI-2 encoded TTSS suggests that these TTSS
3 resulted from separate horizontal gene transfer events, and not gene duplication
4 (Hensel, 2004). SPI-2 is about 40kb, has 42 open reading frames, and is inserted
5 adjacent to the *valV* tRNA gene. SPI-2 is divided into two segments, a smaller 14.5kb
6 containing five *ttr* genes involved in tetrathionate reduction, and a larger 25kb region
7 in which four operons exist, *ssa* (genes encoding the TTSS), *ssr* (secretion system
8 regulator genes), *ssc* (secretion system chaperone genes) and *sse* (secretion system
9 effector genes). SPI-2 genes were found to be stable and present in all clinical strains
10 of *S. enterica*, but not in *S. bongori* (Hensel, 2000). The essential function of the SPI-
11 2 region seems to be in assisting the proliferation of the *Salmonella* infection once
12 invasion has occurred. These functions aid survival in phagocytic cells and the ability
13 to replicate within the *Salmonella*-containing vesicle. SifA (*Salmonella* induced
14 filament protein A), an effector protein encoded on a locus outside of SPI-2
15 translocated by TTSS-2, is an example of a function which is required to maintain the
16 integrity of the phagosomal membrane of the SCV during intracellular proliferation.
17 TTSS-2 is also translocates pipB (pathogenicity island protein B), an effector protein
18 encoded by SPI-5.

19 SPI-2 prevents the co-localization of damaging phagocyte reactive oxygen and
20 nitrogen intermediates (Schmidt and Hensel, 2004). The TTSS encoded by SPI-2 is
21 required for the intracellular survival of *Salmonella* in murine macrophages (Santos et
22 al., 2003). Mutations in SPI-2 genes have resulted in reduced expression of SPI-1
23 genes (Hensel, 2000).

24

1 *Salmonella* pathogenicity island 3, SPI-3 is a 17kb insertion in the *selC* (the tRNA
2 gene for selenocysteine) locus, a locus that is used as an insertion point for
3 pathogenicity islands in *E.coli* (Schmidt and Hensel, 2004). SPI-3 contains *mgtCB*
4 (Magnesium transport ATPase accessory) genes, which are important in the growth of
5 Mg^{2+} limiting environment, and also have been shown to be required for intra-
6 macrophage survival and virulence in mice (Marcus et al., 2000; van Asten and van
7 Dijk, 2005). *mgtCB* genes were found in all *Salmonella* serotypes including *S.*
8 *bongori* (Fierer and Guiney, 2001). Upon examination of SPI-3, in different
9 subspecies and serotypes of *Salmonella*, it was found that this region exhibits
10 extensive structure variations in the form of deletions and insertion of additional gene
11 clusters. The central SPI-3 region genes are flanked by remnant IS elements,
12 signifying an insertion within this SPI region (Hensel, 2004). Other putative virulence
13 genes which have been identified on this stretch of DNA are *misL* (an extracellular
14 matrix adhesion involved in intestinal colonization), similar to the AIDA-1 adhesin of
15 EPEC, and the putative transcriptional regulator *marT*, similar to *Vibrio cholerae*
16 ToxR. The roles of these putative genes in the virulence of *Salmonella* have not been
17 determined (Schmidt and Hensel, 2004).

18

19 *Salmonella* pathogenicity island 4, SPI-4 is a 25kb DNA insertion, located beside *ssb*,
20 which encodes a single stranded DNA binding protein and *soxSR*, which encodes
21 superoxide response regulatory genes (Marcus et al., 2000). SPI-4 has 18 putative
22 ORFs, is thought to encode a type one secretion system that mediates toxin secretion
23 and is thought to contain a gene important in survival in macrophages (van Asten and
24 van Dijk, 2005; Schmidt and Hensel, 2004). The main functions of SPI-4 in
25 *Salmonella* have yet to be elucidated.

1

2 *Salmonella* pathogenicity island 5, SPI-5 is a 7kb locus, and is located between *serT*,
3 which encodes a serine tRNA and *copS/copR*, a copper inducible two-component
4 regulatory system. A mutation in some of the regions of SPI-5 causes attenuation of
5 the enteritis response. This leads to the implication that these genes are required for
6 enteropathogenesis (Marcus et al., 2000). SPI-5 harbours the gene for *sopB*
7 (salmonella outer protein B), an effector protein of the SPI-1 encoded TTSS, and also
8 *pipB* , an effector protein of the SPI-2 encoded TTSS (Schmidt and Hensel, 2004).

9 .

10 SPI-6 is a 59kb insertion beside the *aspV* tRNA gene for selenocysteine, encoding the
11 fimbrial *saf* (*Salmonella* atypical fimbriae) gene cluster, *pagN*, encoding an putative
12 invasion, and many other genes of unknown function.

13

14 SPI-7 is specific for serovars Typhi, Dublin and Paratyphi C. It is 133kb, and is
15 inserted beside the tRNA gene for phenylalanine, *pheU*. SPI-7 encodes the Vi antigen,
16 a capsular exopolysaccharide, the *sopE* phage effector protein, as well as the putative
17 virulence *pil* gene cluster encoding a type IV pilus. This large pathogenicity island
18 was shown to be excised spontaneously in a hospital setting (Hensel, 2004). The
19 pathogenicity determinants encoded by SPI-7 have been shown to play an important
20 role in epithelial cell invasion by *S. Dublin* (Bueno et al., 2004). Although the transfer
21 of SPI-7 has not been demonstrated as yet, it is thought of as an unstable genetic
22 element, or an integrative conjugative element (ICE) (Burrus and Waldor, 2004), and
23 the spontaneous precise excision of this component has been shown by Bueno et al.
24 (2004).

25

1 SPI-8 is 6.8kb long and located beside *pheV*, which is the structural gene for tRNA^{Phe}.
2 Bacteriocins have been putatively recognised as virulence factors encoded in this
3 region so far. An integrase encoding gene has been recognised on SPI-8, indicating the
4 mobility of this element, but so far it has only been found to be specific for serovar
5 Typhi. The 16 kb SPI-9 encodes a type I secretion system, and a single RTX (Repeats
6 in Toxin)-like protein, which may be putative virulence factors (Hensel, 2004). SPI-
7 10, a large 32.8kb insertion, located at *leuX* tRNA, the structural gene for tRNA^{Leu},
8 encodes the *sef* (*Salmonella enteritidis* fimbriae) fimbrial operon and also a cryptic
9 bacteriophage (Hensel 2004).

10 **6.6 Conclusion**

11 *Salmonella* contain many mobile genetic elements, part of the accessory
12 genome, which are responsible for the genetic diversity and ecological diversity of this
13 species (Porwollik and McClelland, 2003). The differences between pathogenicity of
14 particular serovars can be attributed to the plasmids and phages encoded by the
15 organism (Fluit, 2005). Evolution of *Salmonella* into a facultative intracellular
16 pathogen is mainly attributed to the acquirement of the pathogenicity islands which
17 confer virulence (Groisman and Ochman, 1997). Different strains of *Salmonella* are
18 adapted to environmental niches, and this is due to the presence of fitness factor-
19 encoding mobile genetic elements. The ability of these elements to transfer
20 horizontally between species and even genus ensures that new strains of pathogenic
21 bacteria will emerge, especially in such cases where there is a high density of bacteria.

22

1 **7. *Staphylococcus aureus***

2 It has been proposed that bacterial genomes are made up of two different regions: the
3 core region, which consists of housekeeping genes and all essential gene functions;
4 and the flexible gene pool, which confers on the bacterium different traits which may
5 be beneficial under certain circumstances (Dobrindt et al., 2004). The genome of *S.*
6 *aureus* is no different, with core genes defined as being present in 95% of isolates and
7 the flexible genes defined as being present in 5% of isolates (Lindsay and Holden,
8 2004). The core genome makes up about 75% of the *S. aureus* genome with the
9 accessory genome accounting for 25%. Comparison of the complete genome
10 sequences between *S. aureus* and *S. epidermis* strains yielded a core set of 1,681
11 genes common to all strains, with most of the unique genes accounted for by the
12 presence or absence of prophage or genomic islands (Gill et al., 2005). The accessory
13 genome consists of mobile or previously mobile genetic elements that can transfer
14 laterally between strains consisting of bacteriophages, pathogenicity islands,
15 chromosomal cassettes, genomic islands, plasmids and transposons. The role of each
16 of these elements in the evolution of *S. aureus* as a food-borne pathogen will be
17 discussed in this document.

18

19 **7.1 *Role of bacteriophages***

20 Temperate bacteriophages in *S. aureus* are common; most strains will carry at least
21 one on their genome. The enterotoxins associated with *S. aureus* food-borne
22 intoxication Staphylococcal Enterotoxin A(SEA) and Staphylococcal Enterotoxin E
23 (SEE) (Balaban and Rasooly, 2000; Novick et al., 2001) are part of temperate
24 bacteriophage and as yet have not been shown to be transferred between strains.

1 Phage conversion has important implications for the evolution of virulent *S. aureus*
2 strains, and generalised transducing phages may be responsible for most of the
3 horizontal gene transfer between different *S. aureus* isolates (Lindsay and Holden,
4 2004). Panton-Valentine leukocidin (PVL) was transferred from a PVL-positive *S.*
5 *aureus* strain to a PVL-negative *S. aureus* strain via phage conversion, using a novel
6 temperate phage ϕ SLT (Narita et al., 2001). Prophages tend to be induced by
7 environmental conditions and it has been found that antibiotics which induce the SOS
8 response e.g. trimethoprim: which prevents the incorporation of thymine into bacterial
9 DNA; and ciprofloxacin: which blocks the replication fork movement by trapping
10 DNA gyrase on DNA also induce prophages that reside on the *S. aureus* genome
11 (Goerke et al., 2006). The induction of phage and phage genome replication results in
12 an increase in the number of toxin genes produced, leading to improved toxin
13 production (Lindsay and Holden, 2005).

14

15 **7.2 Role of plasmids**

16 *S. aureus* isolates frequently carry one or more plasmids which are either the free
17 form or integrated into the chromosome and are classified based on their size and the
18 genes they carry. These plasmids are most likely transferred by transduction, as
19 conjugative plasmids are relatively uncommon and *S. aureus* is not naturally
20 competent (Lindsay and Holden, 2004). *Staphylococcus* enterotoxin D (SED),
21 *Staphylococcus* enterotoxin R (SER), and *Staphylococcus* enterotoxin J (SEJ) are
22 present on the 27.6kb plasmid pIB485, and therefore may have the capacity to be
23 transferred between strains via conjugation mechanisms, but there is no evidence of
24 this to date.

1

2 ***7.3 Role of insertion sequences***

3 Seven types of insertion sequence elements were identified in the genomes of *S.*
4 *aureus* and *S. epidermis* following complete genome sequencing of both organisms
5 (Gill et al., 2005), these are randomly distributed throughout the genomes of both
6 organisms. The presence of these insertion sequences gives *S. aureus* more potential
7 for gene mobility and exchange.

8

9 ***7.4 Role of pathogenicity islands***

10 *S. aureus* contains numerous pathogenicity islands, *Staphylococcus aureus*
11 pathogenicity islands (SaPI), which comprise part of the flexible genome. To date
12 there have been seven pathogenicity islands described based on particular
13 characteristics of their structure in genome regions (Gill et al., 2005). Pathogenicity
14 islands contain phage related integrase genes, suggesting that they are integrated and
15 excised in a similar fashion to prophages. They are divided into groups depending on
16 insertion site and integrase homology, and no one strain seems to carry more than one
17 copy of each type (Lindsay and Holden, 2004). The prototype pathogenicity island
18 SaPI1 is mobilizable with the assistance of the transducing phages Φ 11 and 80 α
19 (Novick et al., 2001). It is thought that all the related pathogenicity islands are
20 mobilizable in the same way as SaPI1, but this has not been proven to date. Úbeda et
21 al. (2005) reported that the bovine specific pathogenicity island SalPIbov2 is mobile
22 without the assistance of helper phage, and excises spontaneously using
23 staphylococcal integrase protein (Sip).

1 Antibiotics have been shown to induce the SOS response in *S. aureus* resulting in a
2 cascade reaction which promotes the induction of horizontal transfer of pathogenicity
3 island SapIbov1 (Úbeda et al., 2005). It was also shown recently that SOS response
4 activation by β -lactam antibiotics really stimulates the transfer of *S. aureus*
5 pathogenicity islands (Maiques et al., 2006). Some of the Staphylococcal enterotoxins
6 lie on pathogenicity islands and therefore can be transferred to other strains. Great
7 caution should be shown when it comes to the use of SOS response-inducing
8 antibiotics, not only because of the promotion of the spread of antibiotic resistance
9 genes, but also because of the promotion of the induction of prophage and SaPIs.

10

11 ***7.5 Role of Staphylococcal cassette chromosome (SCC)***

12 The genetic determinant of methicillin resistance in *S. aureus*, *mecA* (encoding a
13 penicillin binding protein, PBP2a) is located on the chromosome, on the methicillin
14 resistance Staphylococcal cassette chromosome, SCCmec. Five different types of
15 SCCmec have been defined, based on the combination of the cassette chromosome
16 recombinase (*ccr*) complex and the *mec* complex (Hanssen and Ericson Sollid, 2006).
17 The origin of this element is unknown, but it is thought that there is a common
18 ancestor of this element and that *Staphylococcus sciuri* contained the evolutionary
19 precursor of *mecA* (Couto et al., 1996; Couto et al., 2003). Transfer between
20 Staphylococci is assumed, but so far has not been proven experimentally, but
21 *Staphylococcus epidermidis* is considered to be a reservoir for genetic determinants in
22 a nosocomial setting because of the genetic similarity between the SSCmec in *S.*
23 *aureus* and *S. epidermidis* (Hanssen and Ericson Sollid, 2006).

1 **7.6 Conclusion**

2 *S. aureus* produces a wide array of toxins and virulence factors responsible for a
3 variety of diseases ranging from food poisoning to invasive diseases. These
4 determinants of pathogenicity are positioned on various mobile genetic elements, in
5 particular the pathogenicity islands, some of which have been created as a result of
6 integration and mobilization of prophages between members of this species (Gill et
7 al., 2005). These mobile genetic elements have been transferred, via horizontal gene
8 transfer, between different *S. aureus* isolates leading to the dissemination of virulence
9 factors and to the evolution of isolates with novel pathogenic potential. Enterotoxins,
10 the agents responsible for food poisoning in *S. aureus*, are most likely to be spread
11 due to the transfer of the pathogenicity islands and bacteriophages which encode these
12 determinants.

13 **8. *Listeria monocytogenes***

14 The *L. monocytogenes* genome does not consist of a plethora of mobile genetic
15 elements as in other bacterial pathogens. Virulence in *L. monocytogenes* is mainly due
16 to the presence of the virulence gene cluster LAPI-1 (*Listeria* pathogenicity island 1)
17 and some accessory islets that encode for the many internalins (protein products of a
18 family of virulence factors). Recently, a response regulator, VirR, was discovered that
19 has a major role in virulence, strains deficient in VirA activity proved to be impaired
20 in pathogenicity (Mandin et al., 2005).

21

22 Genomic differences between *L. monocytogenes* and non-pathogenic *L. innocua* have
23 been attributed to the three mechanisms of horizontal gene transfer (conjugation,
24 transformation and transduction) (Buchrieser et al., 2003). The role of mobile genetic

1 elements in the evolution and transfer of virulence genes in *L. monocytogenes* will be
2 discussed below.

3 **8.1 Role of bacteriophages**

4 Thus far, bacteriophages have been shown not to play a major part in the acquisition
5 of virulence genes of *Listeria monocytogenes* (Buchrieser et al., 2003). Generalized
6 transduction, however, where any gene within a donor can be transferred to a recipient
7 strain by lytic or temperate bacteriophage, has been demonstrated in *L.*
8 *monocytogenes* by Hodgson et al. (2000). This was the first report of generalized
9 transduction in this species and a number of listerial bacteriophages were shown to be
10 capable of generalized transduction. However, these bacteriophages have narrow host
11 ranges, so the chance of transferring genes to other serotypes or other species seems
12 quite low. Comparative genome analysis of five *Listeria* strains (*L. monocytogenes*
13 F2365 –serotype 4b, *L. monocytogenes* F6854-serotype 1/2a, *L. monocytogenes*
14 H7858-serotype 4b, *L. monocytogenes* EDG-e -serotype 1/2a and *L. innocua* strain
15 CLIP 11262) revealed the presence of nine putative prophages and five defective or
16 satellite prophages on the *Listeria* genome (Nelson et al., 2004).

17 **8.2 Role of plasmids**

18 pLM80, an 80 kb plasmid found in *L. monocytogenes* H7858, contains several
19 different transposable elements that are not found on the chromosome, leading to the
20 conclusion that this plasmid is a recent acquisition in this particular serotype of *L.*
21 *monocytogenes* (Nelson et al., 2004). A possible transfer apparatus is encoded on this
22 plasmid, which may play a role in plasmid transfer in this organism. However, the
23 mechanisms of plasmid transfer in *Listeria* species have thus far not been explained.

24

1 **8.3 Role of insertion sequences**

2 From comparison between the completed genome of *L. monocytogenes* and *L.*
3 *innocua*, it was evident that neither of these genomes contained many IS elements,
4 which suggests that lateral gene transfer via IS elements has not occurred to any great
5 significance in *Listeria* species (Buchrieser et al., 2003). Comparison between
6 genome sequences of *Listeria monocytogenes* serotype 4b strains F2365 and H7859
7 revealed the absence of insertion sequences, but revealed the presence of some copies
8 of the transposase ORFA (open reading frame A). It is thought that this transposase
9 was present in the ancestral *Listeria* before the divergence of the different strains
10 (Nelson et al., 2004). Virulence determinants previously described by Vazquez-
11 Boland et. al. (2001a, 2001b) do not have IS elements associated with them, therefore
12 it can be thought that they have not been transferred by IS elements.

13 **8.4 Role of pathogenicity islands**

14 Pathogenicity in *Listeria* species is dependent on the presence of a 9kb virulence
15 cluster which contains six physically linked genes (Schmidt and Hensel, 2004). This
16 virulence cluster is described as *Listeria* pathogenicity island 1 (LAPI-1), and is
17 essential for intracellular parasitism (Vazquez-Boland et al., 2001a). Pathogenic
18 *Listeria* species, *L. monocytogenes* and *L. ivanovii* and non-pathogenic species *L.*
19 *seeligeri* all contain some form of this virulence cluster Even though the cluster in *L.*
20 *seeligeri* contains five additional genes, this strain is not pathogenic, it is missing
21 some yet undefined element of pathogenicity (Schmid et al., 2005).. The degree of
22 divergence of this cluster in the different species suggests that a virulence cluster with
23 similar structure was present in a common ancestor of all *Listeria* species (Vazquez-
24 Boland et al., 2001a). The pathogenicity of *L. monocytogenes* centres around LAPI-1,

1 which contains six virulence factors: *prfA*, *plcA*, *hly*, *mpl*, *actA* and *pclB*. This 9kb
2 genomic region, coined an “intracellular life” gene cassette, is stably inserted at the
3 same position in the chromosome of *Listeria* species, and is integral for the
4 pathogenicity potential of *L. monocytogenes* (Vazquez-Boland et al., 2001b).

5
6 The evolution of the pathogenic lifestyle of the genus *Listeria* has been examined by
7 Schmid et al. (2005) They phylogenetically analyzed housekeeping genes that flank
8 the virulence gene cluster: *ldh* (encoding lactate dehydrogenase), *prs* (encoding a
9 pyrophosphate synthetase) and *vcIB* (encoding a conserved protein of unknown
10 function; the 16S and 23S rRNA, and the *iap* (encoding p60, a major extracellular
11 protein) genes in the six species of *Listeria*. Since the virulence gene cluster is
12 inserted in the same position in all of the carrying *Listeria* strains and no obvious trace
13 of lateral gene transfer was found such as: mobility genes, insertion sequences, direct
14 repeats or target sequences (Schmidt and Hensel, 2004) it is likely that a common
15 ancestor possessed this gene cluster and passed it on vertically to its descendants. This
16 cluster was then lost in two independent events in the two non-pathogenic species *L.*
17 *innocua* and *L. welshmeri*.

18
19 LAPI-1 does not fit the typical definition of a pathogenicity island as the G+C
20 content, codon usage and dinucleotide frequency does not differ from the rest of the
21 genome (Vazquez-Boland et al., 2001a). All of this information serves to further
22 verify the stated idea that the virulence gene cluster has evolved within a common
23 ancestor of the current *Listeria* species.

24

1 The soil was probably the main habitat of the common *Listeria* ancestor, it is thought
2 that the virulence gene cluster evolved as a defence against phagocytosis by soil
3 protists, and it changed further as a result of constant contact with vertebrate hosts,
4 into its functions adding to intracellular parasitism (Vazquez-Boland et al., 2001a).
5 Genes which confer defence against phagocytosis include: *hly*, which encodes a pore-
6 forming toxin, essential for disruption of the phagocytic vacuole, *actA*, which encodes
7 the surface protein ActA, the factor responsible for actin-based motility and cell-to-
8 cell spread, and *plcB*, encoding protein PlcB which cooperates with Hly protein in the
9 disruption of the phagocytic vacuole.

10

11 Where this virulence cluster originated from is a cause of conjecture. The low G+C
12 genetic composition suggests that the donor was in a similar Gram-positive
13 phylogenetic division to *Listeria*. Some genes in this cluster conform to this idea, e.g.
14 *hly*, which encodes a protein that is a member of a broad family of pore-forming
15 toxins only distributed in low G+C Gram-positive bacteria. However, there is some
16 thought that these genes were acquired by horizontal transfer from eukaryotes, e.g.
17 ActA, the surface protein responsible for actin based motility, does not have any
18 homology with prokaryotic proteins, but some domains of this protein mimic
19 functional motifs that are present in eukaryotic proteins (Vazquez-Boland et al.,
20 2001a). Whether this cluster was acquired as a complete cassette in one recombination
21 event, or as a result of many assembly steps is also questionable, but the presence of
22 open reading frames with some sequence similarities to transducing bacteriophage
23 suggest that the whole cluster belongs to a genetic element that was once mobile.

1 **8.5 Role of internalin islets**

2 The internalins, proteins that have a characteristic domain containing a variable
3 number of leucine-rich repeats (LRRs), are present on a number of different loci on
4 the *L. monocytogenes* genome. These loci form a multigene family that is exclusive to
5 *Listeria*, responsible for mediating protein-protein interactions. These proteins are
6 involved with the entry of *Listeria* into particular cell types (Dussurget et al., 2004).
7 The diverse *inl* genes form up to several internalin islets, and have a high degree of
8 sequence similarity. This similarity makes the internalins naturally disposed to
9 recombination, shown by the fact that the same internalin islet had a different
10 complement of *inl* genes in two different isolates of the same serovar of *L.*
11 *monocytogenes* (Vazquez-Boland et al., 2001a). It is postulated, therefore, that the
12 multigene internalin family arose from a single internalin gene, in the *Listeria*
13 ancestor, by gene duplication and sequence exchanges by homologous recombination
14 events.

15

16 **8.6 Role of conjugative transposons**

17 Tetracycline resistance determinants have been detected in *Listeria* species from
18 human and food-processing origins at a low incidence, and these determinants have
19 been inferred to be encoded on a conjugative element of the Tn916-Tn1545 family,
20 from experimental evidence of transfer of tetracycline resistance to *Enterococcus*
21 *faecalis*. Sequencing data analysis suggests that *tet(M)* acquisition, resulting in
22 tetracycline resistance, could be the result of transfers between other Gram-positive
23 organisms (Bertrand et al., 2005).

1 **8.7 Conclusion**

2 It is thought that an ancestor of *Listeria* species contained a virulence gene cluster
3 with a similar structure to LAPI-1 (Vazquez-Boland et al., 2001a), that was lost in two
4 independent events in *L. innocua* and *L. welshimeri* (Schmid *et al.*, 2005), and
5 evolved in the other *Listeria* species. This gene cluster may be an ancient PAI
6 acquired by a common *Listeria* ancestor which has become stabilized in the core
7 genome of pathogenic *Listeria* . This gene cluster contains two open reading frames,
8 which display considerable similarities with bacteriophage proteins, suggesting that it
9 may have been originally mobilized by bacteriophage transduction. Internalins,
10 proteins that are essential for protein-protein interactions, are also required for
11 virulence of *Listeria* species (Vazquez-Boland et al., 2001a). The fact that all *Listeria*
12 species contain internalin genes suggests that these genes were present in the common
13 *Listeria* ancestor and are as old as LAPI-1. But the different types of internalin genes
14 in each *Listeria* strain implies that horizontal gene transfer has played a part in the
15 diversification of the internalin multigene family, perhaps catalyzed by bacteriophage
16 transduction. In the past few years, the genomes of different *L. monocytogenes* and
17 *Listeria* strains have been completely sequenced. Comparison of these genomes will
18 lead to further insights into the evolution of *L. monocytogenes* pathogenicity.

19

20 **9. Overall Conclusions**

21 Bacterial genomes are mosaic structures of genetic information that are in a constant
22 state of flux. Mobile genetic elements serve as the catalyst for the transfer of genes
23 between different organisms known as horizontal gene transfer. The evolution of

1 pathogenic organisms has been influenced by this exchange of different mobile
2 genetic elements.

3

4 The acquisition of pathogenicity islands, discrete elements of the genome that are
5 characterised by differences in G+C content and codon usage, has been a main source
6 of conversion of a species from a non-pathogenic species to a pathogenic species.
7 Pathogenicity islands allow evolution to occur in quantum leaps, in decades rather
8 than centuries, and enable the recipient organism to reside in a particular ecological
9 niche. Examples include: *L. monocytogenes* and LAPI-1, the pathogenicity island that
10 allows for intracellular parasitism (Vazquez-Boland, 2001); the LEE of
11 Enteropathogenic *E. coli* (including *E. coli* O157:H7), which is responsible for
12 attaching and effacing lesions; SPI-1 and SPI-2 from *Salmonella*, responsible for
13 invasion, enteropathogenesis and intracellular pathogenesis, respectively; and the
14 various SAPI islands from *S. aureus*, which harbour enterotoxins and other toxin
15 genes (Schmidt and Hensel, 2004)

16

17 Prophages have also played a major role in the deployment of virulence factors
18 between pathogenic and non-pathogenic organisms. For each of the different food-
19 borne pathogens examined in this report, bacteriophages have played a part in forming
20 their genomic architecture, and also in the evolution of these organisms as pathogens.

21

22 Other mobile genetic elements have proven to be significant in the evolution of
23 virulence relevant to food-borne infection and intoxication including: virulence
24 plasmids in *Salmonella*, internalin islets in *L. monocytogenes*.

25

1 Organisms that share ecological niches are more likely to exchange genes than
2 organisms that do not (Barkay and Smets, 2005), e.g. archaeal genes were found in the
3 genome of the bacterium *Thermotoga maritima*, and bacterial genes were found in the
4 archaeal *Methanosarcinia mazei*. Horizontal gene transfer has recently been found to
5 have increased support in areas of high bacterial density, energy and diversity such as,
6 biofilms, solid-liquid-gaseous interfaces and the external and internal surfaces of
7 plants and animals.

8

9 Incorporation of virulence genes by horizontal gene transfer poses a problem for the
10 recipient organism as the obtained sequences will only be effective if expression is
11 coordinated with that of the rest of the genome, therefore, many of the genes present
12 on mobile genetic elements are controlled by regulatory systems that were inherently
13 present in the recipient genome already (Groisman and Ochman, 1997).

14

15 A novel approach to the study of horizontal gene transfer was carried out by Susanna
16 et al. (2006), where the transcription activator ComK from *Bacillus subtilis*, was
17 transferred into *Lactococcus lactis*. In *B. subtilis*, ComK activates transcription by
18 binding to K-boxes in the upstream of ComK-dependent genes. *L. lactis* contains a
19 number of putative K-boxes which were thought of as being potential targets for
20 regulation by ComK. The predicted regulation of those specific genes with *B. subtilis*
21 ComK in *L. lactis* was not found, showing that horizontal gene transfer does not
22 always deliver the expected functionality of a gene from one species to another.

23

24 Studying evolutionary effects of pathogenic bacteria gives us an understanding as to
25 why some pathogens cause more harm than others and the environmental

1 circumstances that afford this harm. This also allows us to grasp the idea that certain
2 human activities advance or prevent this harm (Ewald, 2004).

3

4 This review has discussed the evolution of certain pathogens by horizontal gene
5 transfer, in the context of potential donors. The next step is to evaluate the potential
6 recipients of the discussed factors and to speculate on the evolution of new pathogens
7 based on past experience.

8

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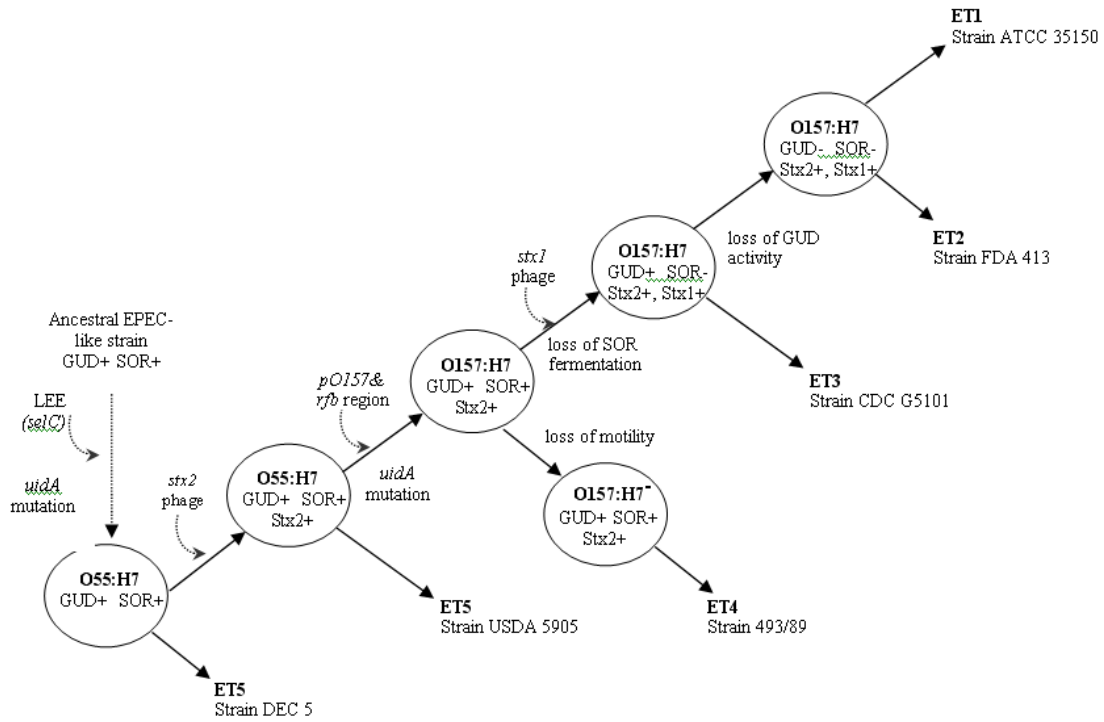
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1 **TABLES**

2 **Table 1.** A proposed model for the evolution of *E. coli* O157:H7 (taken from Feng et.
3 al., 1998). Based on mutations in *uidA*, *stx* production, SOR and GUD phenotypes,
4 and multilocus enzyme electrophoretic profiles of *E. coli* O157:H7 and its relatives.



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- 1 **Table 2.** Overview of the ten known pathogenicity islands of Salmonella (adapted
- 2 from Hensel, 2004)

Designation (alternative)	Size ^a in kb	Base composition %G+C (range)	Insertion point	Virulence functions
SPI-1	39.8	47	<i>flhA-mutS</i>	T3SS, iron uptake
SPI-2	39.7	44.6	tRNA <i>valV</i>	T3SS
SPI-3	17.3	47.3 (39.8–49.3)	tRNA <i>selC</i>	Mg ²⁺ uptake
SPI-4	23.4	44.8	(tRNA like)	Unknown
SPI-5	7.6	43.6	tRNA <i>serT</i>	T3SS effectors
SPI-6	59	51.5	tRNA <i>aspV</i>	Fimbriae
SPI-7)	133	49.7 (44–53)	tRNA <i>pheU</i>	Vi antigen, pilus assembly, <i>sopE</i>
SPI-8	6.8	38.1	tRNA <i>pheV</i>	Unknown
SPI-9	16.3	56.7	prophage	Putative toxin, unknown
SPI-10	32.8	46.6	tRNA <i>leuX</i>	Sef fimbriae

3