

1 **|Horizontal gene transfer of virulence**
2 **determinants in selected bacterial**
3 **foodborne pathogens**

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11 **Key words:** Horizontal gene transfer, *E. coli* O157:H7, *L. monocytogenes*, *S. aureus*,
12 *Salmonella*

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17 *Abbreviations:* °C, degrees Celsius; DNA, deoxyribonucleic acid; EHEC,
18 enterohemorrhagic *E. coli*; HGT, horizontal gene transfer; Inc, incompatibility; MDR,
19 multidrug resistant region; MRSA, methicillin resistant *Staphylococcus aureus*; SaPI,
20 *Staphylococcus aureus* pathogenicity island; SCC, staphylococcal cassette
21 chromosome; SGI, *Salmonella* genomic island; STEC, shiga toxin-producing *E. coli*;
22 Stx, shiga toxin;

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Abstract

This review describes horizontal gene transfer from a historical point of view, with descriptions of the first instances of the different bacterial transfer mechanisms: conjugation, transduction and transformation, as well as examples of some of the early acknowledged transfer events. Gene transfer from four selected foodborne pathogens: *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella* are highlighted.

1 **Introduction**

2 Horizontal Gene Transfer (HGT) provides novel functions which may allow recipient
3 organisms to proliferate in a previously unexploited niche, leading to diversification
4 of natural populations (Virdi and Sachdeva, 2005). Previous reviews by Kelly et. al.
5 (a, b, this issue) examined four foodborne pathogens: *Escherichia coli*, *Listeria*
6 *monocytogenes*, *Staphylococcus aureus* and *Salmonella* spp and in respect to the
7 virulence factors they contain as well as the role different mobile genetic elements
8 play in the evolution of these organisms. This review gives a brief historical account
9 of the different genetic transfer mechanisms in bacteria, how transfer events were first
10 discovered and how they were first achieved *in vitro*. The transfer of genes from the
11 same four foodborne bacterial pathogens is examined, and future transfer events are
12 considered.

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14 There is no doubt whatsoever about the importance of HGT in the architecture and
15 constant evolution of bacterial species. In order for virulence genes to be transferred
16 from a pathogenic species to a non-pathogenic recipient strain, and actually to be used
17 in the original virulent capacity, there are a number of conditions that need to be
18 fulfilled. Many barriers exist against the efficient transfer, uptake and stabilization of
19 extraneous DNA. The ideal situation is where genes with a certain function, travel
20 from donor to recipient by whatever means necessary, and end up expressing the same
21 function in the recipient cell as in the original donor cell (Thomas and Nielsen, 2005).

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23 The concept of horizontal gene transfer (HGT) was first supported by the transfer of
24 virulence determinants between pneumococci in infected mice, a phenomenon

1 discovered by Griffith (1928), which later became known as transformation. Since
2 then gene transfer mechanisms have been found to be ubiquitous in bacteria. As
3 discussed in a previous review (Kelly et al., a this issue), there are many ways that
4 gene transfer occurs: transformation, transduction and conjugation. The transfer of
5 DNA by vesicles as another method of gene transfer was discovered for bacteria and
6 especially for *E. coli* O157:H7 recently (Kolling and Matthews, 1999, Yaron et al.
7 2000). The advent of genome sequencing technology and the completion of genome
8 sequences from many bacteria has lead to comparisons between genomes.
9 Phylogenetic comparison, where the similarity or dissimilarity of genomes are
10 compared, and parametric comparison, where the total genome of an organism is
11 examined and genes that appear to be atypical to the rest of the genome (by GC
12 content for example) are two approaches that are used to determine whether
13 horizontal gene transfer events have occurred in a genome (Lawrence and Ochman,
14 2002). From this sequencing data horizontal gene transfer events have found to be rife
15 in most species examined thus far, an exception being *Buchnera aphidicola* which has
16 not had any gene rearrangements or gene acquisitions in over 50 million years (Tamas
17 et. al., 2002). From a laboratory perspective gene transfer has been demonstrated
18 between a plethora of bacterial species and genera. Gene transfer has also been shown
19 between bacteria to different kingdoms, as in the case of *Agrobacterium tumefaciens*
20 and the transfer of the Ti plasmid to plants and yeast (Davison, 1999). The transfer of
21 DNA from plants to bacteria has also been documented as in the case of a transgenic
22 plant conferring kanamycin resistance to an *Acinetobacter* strain (Gebhard and
23 Smalla, 1998).

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1 Whether a particular gene is transferred successfully depends on the type of transfer,
2 the relationship between the molecule being transferred with the type of transfer, and
3 on other factors, such as the distribution of integrases, specific enzymes concerned
4 with the integration of DNA (Eede et al., 2004). Genes acquired by horizontal transfer
5 from any species can be deleterious, neutral or beneficial to the recipient strain. Genes
6 that are deleterious are usually removed by selection, neutral genes could possibly be
7 detained and genes that have a beneficial effect are selected for (Bolotin *et al.*, 2004).

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9 **Gene transfer processes**

10 HGT processes were originally detected experimentally about 50 years ago. During
11 the last two decades, conjugation, transformation and transduction have been
12 identified in many bacterial species in a variety of bacterial habitats ranging from soils
13 to biofilms to the gastrointestinal tract (de Vries and Wackernagel, 2004).

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15 ***Conjugation***

16 For conjugation, gene transfer mediated by cell-to-cell contact, the donor and
17 recipient strains need to be in the same environment to allow for the creation of the
18 conjugative machinery between the donor and recipient cells. The virulence genes in
19 question need to be transferable, i.e. they need to exist on mobile genetic elements,
20 segments of DNA encoding proteins important for the mediation of movement of
21 DNA within genomes, as discussed previously (Kelly et. al., a, this issue). The
22 transported DNA has to avoid the restriction nucleases of the recipient and these
23 genes need to be recombined efficiently in order for effective future persistence. The
24 recipient has the more active role that promotes the uptake of exogenous DNA in

1 transformation but with conjugative methods, the donor is the more active participant
2 in the DNA transfer process (Thomas and Nielsen, 2005). The majority of bacterial
3 gene transfer events in the environment are via conjugation. There are many types of
4 conjugative events, which all have been shown to occur in natural conditions. Many
5 conjugative plasmids and transposons have been shown to have a broad host range
6 including most Gram-negative bacteria and even some Gram-positive bacteria. RK2 is
7 an example of a plasmid capable of conjugal transfer between most gram negative
8 bacteria (Thomas, 1981). This plasmid has been used to create plasmid vectors which
9 have been transferred from Gram-negative *E. coli* to many Gram positive bacterial
10 strains (Trieu-Cuot et al. 1987). Incompatibility group P (IncP) plasmids can transfer
11 by conjugation into Gram-positive, Gram-negative bacteria and *Saccharomyces*
12 *cerevisiae* (Thomson et al., 1993). Many diverse environments ranging from raw
13 salmon cutting boards and the gut of soil micro-arthropods to *in planta* with citrus
14 trees have been privy to horizontal gene transfer via conjugation mechanisms
15 (Hoffmann et al., 1998, Davison, 1999, El Yacoubi et. al., 2007). Conjugal transfer of
16 resistance plasmids was demonstrated *in vitro* in the 1970s by many groups including:
17 Lakhota et al (1972); Nivas et al.(1976), where R factors (plasmids which encode
18 resistance determinants) were transferred between two Gram negative strains: *E. coli*
19 and *Salmonella*. *In vitro* conjugative transfer between Gram-positive *Lactobacillus*
20 *acidophilus* and *Lactobacillus reuteri* was proven by Vescovo et al. (1983). Droge et
21 al. have extensively discussed the role conjugation plays in horizontal gene transfer
22 with regards to biosafety, as this process is the most promiscuous in nature, (Droge et
23 al., 1998).

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1 Plasmids can be classified into what is known as incompatibility (Inc) groups, first
2 introduced by Novick (1987). This Inc scheme is based on the introduction of a
3 plasmid with an unknown Inc group, by conjugation or transformation, into a strain
4 carrying a plasmid with a known Inc group. If the resident plasmid is eradicated from
5 the progeny, then the unknown plasmid has the same Inc group as the resident
6 plasmid. Plasmids with the same replication mechanisms are “incompatible”, and
7 plasmids with different replication mechanisms are “compatible”. Therefore, two
8 plasmids belonging to the same Inc group cannot proliferate in the same cell line
9 (Carattoli et al., 2005). Plasmids with diverse Inc groups differ in host range of
10 transfer, autonomous replication, pilus structure and size (Droge et al., 1998). This is
11 important when considering the dissemination and evolution of plasmids conferring
12 resistances or virulence determinants into strains which may have indigenous
13 plasmids present.

14

15 ***Transformation***

16 Many bacterial species are naturally transformable, i.e. they have the natural ability to
17 take up naked DNA from the environment. In 1994 Lorenz and Wackernagel (1994)
18 listed 44 bacterial strains as naturally transformable; this list was updated by de Vries
19 and Wackernagel in 2004 to make almost 90 species described as naturally
20 transformable to date. This does not include the transformation abilities of the many
21 unculturable bacteria that exist in a range of environments. These naturally
22 transformable strains exist in many different niches and have many different
23 physiologies. Transformation has been shown to occur in many natural ecosystems
24 ranging from soil microcosms to river or spring water. The level of transformability
25 and competence varies between strains of a species, and therefore the fraction of

1 transformable bacteria present is more than likely underestimated (de Vries and
2 Wackernagel, 2004). The exogenous DNA must have certain features in order to be
3 successfully integrated into the host genome. Some naturally competent bacteria such
4 as *Bacillus subtilis* and *Acinetobacter* take up exogenous DNA from any source with
5 the same efficiency, whereas bacteria such as *Neisseria gonorrhoeae* and
6 *Haemophilus influenzae* take up DNA preferentially from their own or related species.
7 The size of the DNA associating with the cell is important, with high molecular
8 weight DNA being the best at effecting transformation. The DNA may be modified by
9 restriction enzymes if it originates from a genetically distant source from the recipient
10 organism. Effective integration of the extraneous DNA occurs by homologous
11 recombination and the more divergent the sequences, the less frequent the
12 homologous recombination events (de Vries and Wackernagel, 2004). Bacterial
13 species which are not naturally transformable need to be made competent by artificial
14 means. This was first achieved chemically by treating bacteria to ice-cold solutions of
15 CaCl_2 and heating the DNA to 37°C or 42°C (Mandel and Higa, 1970, Cohen, 1972).
16 The present day methods for making bacterial cells competent are variations based on
17 these original techniques, variations which include the use of: divalent cations;
18 different buffers; harvesting the cells at different stages of the growth cycle; changing
19 the extent and temperature of heat shock; to name but a few (Sambrook and Russell,
20 2001). Cells can also be made competent by physical means such as electroporation,
21 where the cells are exposed to an electrical charge destabilizing the cell membranes
22 and inducing the formation of transient membrane pores through which DNA
23 molecules can pass. Originally, this method was used for eukaryotic cells, but was
24 subsequently adapted for transforming *E. coli* and other bacteria by plasmids (Dower
25 et al., 1988, Miller, 1988). This method is thought of as the easiest, most efficient and

1 most reproducible method for transformation of bacterial cells with DNA (Sambrook
2 and Russell, 2001). In transformation, where exogenous DNA is taken up directly
3 from the environment, integrated and expressed under natural growth conditions,
4 DNA must firstly remain intact by avoiding degradation from nucleases in the
5 environment; it must be actively taken up by competent bacteria and then successfully
6 recombined into the host genome to ensure efficient persistence in future generations
7 (Thomas and Nielsen, 2005).

8

9 ***Transduction***

10 Transduction, bacteriophage-mediated gene transfer, is a specific HGT process with a
11 limited host range, described as a significant factor in the evolution of bacteria
12 (Brabban et al., 2005). Many bacterial species contain a number of prophages that
13 encode various virulence factors: *E. coli* O157:H7 contains Stx1 and Stx2 prophages,
14 which confer the bacterium with Shiga-toxin producing capabilities (Wick et. al.,
15 2005); bacteriophage-encoded functions enabling *Salmonella* to invade tissues and
16 avoid immune responses are encoded by bacteriophages (Boyd and Brussow, 2002);
17 and Staphylococcal Enterotoxin A (SEA) and Staphylococcal Enterotoxin E (SEE) of
18 *S. aureus* are associated with a temperate bacteriophage (Balaban and Rasooly, 2000;
19 Novick et al., 2001). The barriers to transduction are similar to the barriers mentioned
20 for conjugation and transformation. The very specific nature of the bacteriophage
21 interaction also serves as a barrier for efficient HGT between different bacterial
22 species.

23

24 Bacteriophages (phages) are common in the environment, and can transfer genes by a
25 generalized or a specialized transduction process. Transduction in the environment

1 was thought to be unlikely due to the highly specific nature of the host range of
2 bacteriophages. Some bacteriophages can exist as lysogens, where the phage DNA
3 integrates with the host DNA and lies dormant as a part of the host genome until
4 induced back to the infective lytic cycle by some environmental signal. These
5 lysogenic phages are protected from degradation by the host cell in which they reside.
6 The DNA protection offered by the phage protein coat in lytic phages also confers
7 relative stability from environmental degradation. Phages have been known as
8 mediators of environmental gene exchange and they also play an integral role in the
9 evolution of new food-borne pathogens (Brabban et al., 2005). Transduction was
10 originally discovered in 1952 by Zinder and Lederberg (Zinder and Lederberg, 1952),
11 while working on transformation in *Salmonella* strains. Bacteriophage λ was used as a
12 cloning vehicle in transduction in the 1970s by a variety of researchers (Sambrook
13 and Russell, 2001), and since then a great deal of λ and non- λ derived phage vectors
14 have been described and used in molecular biology for the orchestrated transfer of
15 genes from strain to strain.

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17 **Transfer of genes from selected donor organisms**

18 The capacity of bacteria to transfer virulence determinants should be a source of
19 concern for the food industry. Transfer of virulence genes from a pathogenic bacterial
20 strain to a non-pathogenic strain may result in the emergence of new foodborne
21 pathogens, and widespread contamination of the food chain. The occurrence of gene
22 transfer in four foodborne pathogens *Escherichia coli*, *Listeria monocytogenes*,
23 *Staphylococcus aureus* and *Salmonella* species will be discussed in this section in

1 order to gain insight into the potential problems that may arise in the future. Some of
2 the transfer events that occur in each bacterium are outlined in table 1.

3

4 ***Escherichia coli***

5 Resistance to tetracycline, sulphonamides, ampicillin and streptomycin are commonly
6 observed in *E. coli* isolates. During the last few years, resistance to clinically relevant,
7 front-line antimicrobials such as fluoroquinolones, extended-spectrum β -lactams
8 (including extended-spectrum cephalosporins) has been emerging among *E. coli*
9 strains (Schroeder et al., 2002). The isolation of antimicrobial-resistant *E. coli* from
10 the intestinal flora of healthy humans and animals, in addition to data which
11 demonstrates that *E. coli* readily transfers plasmids to strains within species or
12 between shiga toxin-producing *E. coli* (STEC, also known as EHEC,
13 enterohemorrhagic *E. coli*) (Johnson et al., 1994) and to strains of different genera
14 such as *Hafnia alvei* (Zhao et al., 2001), indicates that commensal *E. coli* strains are
15 an important reservoir of transferable antimicrobial resistance genes (Singh et al.,
16 2005). With isolates of *Salmonella enterica* serovar Typhi, *E. coli* and *Klebsiella*
17 *pneumoniae* recovered from stool samples it was shown that *E. coli*, *K. pneumoniae*,
18 and *S. Typhi* were all conjugally proficient; antibiotic resistance was transferred to *S.*
19 *Typhi* at high frequencies. Resistance markers were transferred in to *E. coli* K-12 at a
20 frequency of approximately 7×10^{-3} . Similarly, *E. coli* K-12 (containing the R factors
21 from *K. pneumoniae*) was able to transfer resistance to clinical isolates of *K.*
22 *pneumoniae*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa* at a frequency of
23 approximately 3×10^{-3} (Schwalbe et al., 1990).

24

1 Shiga toxin production is almost invariably associated with lamboid stx phages.
2 Phages are considered as highly mobile genetic elements which play a profound role
3 in horizontal gene transfer and the emergence of new STEC pathotypes (Muniesa,
4 2000). The wide distribution of Stx1 and Stx2 variants in different bacteria indicates
5 that these phages possess the ability of transmitting stx genes throughout the
6 *Enterobacteriaceae*, although, several studies reported a limited host range for
7 individual stx phages (Saunders et al., 2001). Stx1- toxin producing strains have been
8 found among *Shigella dysenteriae* type I, *Shigella sonnei* (Strauch et al. 2001), widely
9 distributed in > 100 serotypes of *E. coli* (Karch et al. 1999) and occasionally in other
10 *Enterobacteriaceae* such as *Citrobacter freundii* (Schmidt et al., 1993, Tschäpe et al.,
11 1995), and *Enterobacter cloacae* (Paton and Paton, 1996). Stx phages can be
12 transmitted between different bacteria *in vivo* and *in vitro*. Extra-intestinal
13 transmission of phages is also possible. The presence of infectious shiga toxin phages
14 could be observed in sewage and in faecally contaminated rivers. Phages or lysogenic
15 strains harbouring Stx2 phages might be the natural reservoir of Stx2 genes and
16 lysogenization could be the main cause of the emergence of STEC strains.
17 Lysogenization/conversion processes could take place in food and water and probably
18 inside the human and animal gut. Ingestion of Stx2 phages has been shown to result in
19 the conversion of non-Stx2-*E. coli* strains present inside the gut to Stx2-producing *E.*
20 *coli* strains thus producing new pathogenic strains (Muniesa and Jofre, 2004). Further
21 studies on the ecology and physiology of Stx phages and their hosts are needed to get
22 a better understanding of the mechanisms and evolutionary forces that extend the
23 genetic spectrum of *E. coli* and other bacterial pathogens (Creuzburg et al., 2005). The
24 key role bacteriophage-mediated gene transfer has been discussed by Brabban et al
25 (2005), where they found that Stx-encoding bacteriophage continues to mediate the

1 transfer of virulence genes within the *E. coli* family and beyond and may be a critical
2 factor in the evolution of pathogens and the emergence of new pathogens.
3
4 Morabito et al. (2002) isolated STEC *E. coli* strains from humans, cattle, and food.
5 Strains belonging to serogroups O26, O111, and O157 were examined for
6 susceptibility to several antimicrobial drugs. Integrons (class 1) were found more
7 frequently in strains belonging to serogroups O111 and O26 than in the O157 isolates.
8 DNA sequence analysis showed that most of the integrons contained the *aadA1* gene
9 cassette conferring resistance to streptomycin/spectinomycin alone or associated with
10 the *drfA1* gene cassette conferring resistance to trimethoprim. In a O157:H7 strain,
11 one integron carried the *aadA2* and *dfrA12* gene cassettes, conferring resistance to
12 streptomycin/spectinomycin and trimethoprim, and the open reading frame F (OrfF)
13 encoding unknown functions. Most of the integrons were carried by *Tn21* derivative
14 transposons and were transferable by conjugation to an *E. coli* K-12 strain. Singh et
15 al. (2005) reported that the transfer of integrons (located on plasmids) by conjugation
16 between strains of *E. coli* resulted in transfer of antimicrobial-resistant phenotypes for
17 ampicillin, chloramphenicol, cefalothin, gentamicin, tetracycline, trimethoprim,
18 sulfamethoxazole and streptomycin and facilitated the emergence and dissemination
19 of antimicrobial resistance among STEC in humans and food animals. Horizontal
20 transfer of nonconjugative plasmids has been shown in biofilms between mixed *E.*
21 *coli* strains by Maeda et al. (2006), with transformation being the most likely
22 mechanism of DNA uptake. This may prove important in the transfer and
23 dissemination of plasmid-encoded virulence determinants in natural environments,
24 and also raises questions as to whether this type of DNA transfer is possible between
25 other bacterial species.

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Listeria monocytogenes

L. monocytogenes is the only one of the eight species of *Listeria* classified as pathogenic for humans, causing a highly fatal illness known as listeriosis (Vazquez-Boland et al., 2001). However, in rare cases another species, *Listeria ivanovii*, which is considered specific to ruminants, is pathogenic to humans (Snapir et al., 2006). Foodborne transmission is the main route of acquiring listeriosis. Immunocompromised individuals such as: the elderly, newborns, and people with HIV are more susceptible to the condition than individuals in the fullness of health.

Many strains of *L. monocytogenes* have demonstrated resistance to various antibiotics, with the first multiresistant strain isolated from France in 1988 (Poyart-Salmeron et al., 1990). Conjugal transfer of various plasmids from *Enterococcus* and *Streptococcus* to *Listeria* and between species of *Listeria* has been described in many reviews (Charpentier and Courvalin, 1999). Further evidence of transfer events comes from Bertrand et al. (2005). Sequence analysis of tetracycline resistant determinant *tet(M)* from a variety of *L. monocytogenes* strains infers that the acquisition of this resistant gene has been from successive transfers between other Gram-positive organisms. Generalized transduction, where any gene within a donor organism can be transferred to a recipient strain by lytic or temperate bacteriophage, has been demonstrated in *L. monocytogenes* by Hodgson et al. (2000), suggesting another method for HGT events in *Listeria*.

1 Transfer of the tetracycline resistance gene, *tet(M)*, from a food source of *L.*
2 *monocytogenes* to an *Enterococcus faecalis* strain has been shown by Bertrand et al
3 (2005). The *L. monocytogenes* strain was found not to contain a detectable plasmid,
4 but does possess a member of the transposon Tn916-Tn1545 family. This may
5 indicate that the transfer of *tet(M)* involved movement of a conjugative transposon
6 element. A study by Zhang et al. (2007) also showed transfer of *tet(M)* from *L.*
7 *monocytogenes*, previously isolated from retail foods, to *E. faecalis*.

8

9 Transfer of any other virulence determinant from *L. monocytogenes* has not been
10 documented to date, but Johnson et. al. (2004) found an atypical strain of *L. innocua*
11 that contained genes from the *Listeria* pathogenicity island-1 (LIPI-1), usually found
12 in *L. monocytogenes*. One explanation the authors put forward for this anomalous
13 strain is horizontal gene transfer from *L. monocytogenes* to *L. innocua*. However, a
14 later study by Volokov et al. (2007) deemed it highly unlikely that horizontal gene
15 transfer occurred between the *L. monocytogenes* and the atypical *L. innocua* strain,
16 but that the *L. innocua* strain evolved from an ancestral *L. monocytogenes* strain.

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18 ***Staphylococcus aureus***

19 *S. aureus* contains several pathogenicity islands, which contain virulence determinants
20 and toxin genes. There are seven pathogenicity islands described based on particular
21 characteristics of their structure in genome regions (Gill et al., 2005). Phage related
22 integrase genes are present on these pathogenicity islands, suggesting that they are
23 integrated and excised in a method similar to prophages. SaPI, known as the prototype
24 pathogenicity island, is mobilizable with the assistance of the transducing phages
25 (Novick et al., 2001, Novick, 2003). Transfer of toxin genes by phage conversion or

1 lysogenic bacteriophage is a central means in virulent strain evolution (Ito, 2003). It is
2 thought that all the related pathogenicity islands are mobilizable in the same way as
3 SaPI1, but this has not been proven to date. Úbeda et al. (2003) reported that the
4 bovine specific pathogenicity island SalPIbov2 is mobile without the assistance of
5 helper phage. It has also been shown by Maiques et al. (2007) that SalPIbov2 can be
6 induced to replicate by different staphylococcal phages. Antibiotics have been shown
7 to induce the SOS response in *S. aureus* resulting in a cascade reaction which
8 promotes the induction of horizontal transfer of pathogenicity island SapIbov1 (Úbeda
9 et al., 2005). Recently, it was demonstrated that SOS response activation by β -lactam
10 antibiotics really stimulates *S. aureus* pathogenicity island transfer (Maiques et al.,
11 2006). Prophage mobilization was also shown to occur by inducing the SOS response
12 with ciprofloxacin (Cirz et al., 2007). Some of the Staphylococcal enterotoxins,
13 responsible for food poisoning, reside on pathogenicity islands thus they may be
14 transferred to strains without certain enterotoxins. When using SOS response-
15 inducing antibiotics, prudence should be exercised, not just only because of the
16 promotion of the spread of antibiotic resistance genes, but also because of the
17 promotion of the induction of pathogenicity islands containing virulence
18 determinants. Through genome comparisons Gill et al. (2005) hypothesised a
19 continuing evolution of virulence and resistance determinants in *S. aureus*, and a
20 transition of *S. epidermidis* from a commensal organism to an opportunistic pathogen
21 via the acquisition of extra virulence factors (Gill et al., 2005).

22

23 ***Salmonella***

24 *Salmonella* species contain a wide variety of mobile genetic elements from
25 pathogenicity islands to conjugative transposons, as previously described (Kelly et al.,

1 b, this issue). Pathogenicity islands contain clusters of functionally related genes
2 necessary for virulence in *Salmonella*. Ten *Salmonella* pathogenicity islands (SPIs)
3 (Bishop *et al.*, 2005) and other regions known as “islets” containing only a few
4 virulence genes (van Asten and van Dijk, 2005) have been found in *Salmonella*
5 species to date. Many of the more prominent *Salmonella* virulence determinants are
6 associated with mobile genetic elements and in many cases have been shown to
7 transfer both *in vivo* and *in vitro*.

8

9 It was originally thought that the antibiotic resistant *Salmonella enterica* serovar
10 Typhimurium DT104 was spread clonally in Europe and the United States (Cloeckaert
11 and Schwarz, 2001). But then it was found that *Salmonella* SGI-1, the genomic island
12 which contains the multiple resistance region was present in a wide range of serovars
13 by Levings *et al.* (2005). This element is present on a 13 kb integron which was
14 shown to be transferable. Doublet *et al.* (2005) reported that SGI-1 could be
15 transferred by conjugation between *Salmonella* strains that were SGI-1 negative and
16 to *E. coli*, with the help of plasmid R55, a helper Inc C plasmid. The mobility of this
17 resistance entity more than likely contributes to spread of antibiotic resistance genes
18 between *S. enterica* serovars. Therefore, the dissemination of *Salmonella* genomic
19 island-1(SGI-1), containing the multidrug resistance region (MDR) by horizontal gene
20 transfer, has been proven by the discovery of this element in different serovars of *S.*
21 *enterica* besides *S. Typhimurium* DT104 and *S. Enteritidis* (Velge *et al.*, 2005).
22 Ahmed *et al.* (2007) reported that the Gram-negative bacterium *Proteus mirabilis*
23 contained a variant of SGI-1 which seemed to be integrated at a chromosomal site
24 different to the *attB* site in *Salmonella* spp. Inspired by this finding, Doublet *et al.*
25 (2007) scanned the sequenced genome for SGI-1 homologous integration sites in *P.*

1 mirabilis and found one gene with 70% identity to the *thdF* integration site (*attB*) in
2 *Salmonella*. Moreover, they scanned many bacteria other than *Salmonella* and *E. coli*,
3 including *Shigella* spp, *Legionella pneumophila* and *Klebsiella pneumoniae* and found
4 potential *attB* sites.

5

6 *Salmonella* strains harbour many different temperate bacteriophages, belonging
7 mainly to the P22 family which can facilitate lateral gene transfer by transduction.
8 This transduction mechanism is responsible for a process known as lysogenic
9 conversion, where non-pathogenic strains are converted to pathogenic strains by the
10 addition of lysogenic phages containing virulence factors (Krylov, 2003). Some of
11 these phages can induce lysogenic conversion from one *Salmonella* Typhimurium
12 phage type to another (Mmolawa et al., 2002). Figueroa-Bossi et al. (Figueroa-Bossi
13 et al., 2001) discussed the many prophages in *Salmonella* and demonstrated the ability
14 of three *S. Typhimurium* phages *Gifsy-1*, *Gifsy-2* and *Gifsy-3* to successfully
15 lysogenize serovars other than *S. Typhimurium*. Virulence determinants which lie on
16 resident prophages can also be transferred between the different families of phages
17 which lie on the genome of one *Salmonella* serovar (Miold et al., 2001). It has been
18 shown that *Salmonella* Typhimurium can transfer antibiotic resistance determinants
19 within human epithelial cells by conjugation (Ferguson *et al.*, 2002). One of the
20 worrying trends observed in *Salmonella* recently is the formation of resistance and
21 virulence cointegrated plasmids, where the resistance determinants are encoded on the
22 virulence plasmids, and can be disseminated to other serovars (Fluit, 2005)

23

1 **Conclusion**

2 This review gives a historical account of horizontal gene transfer, illustrating the
3 importance of transfer events in the emergence of new bacterial strains, and also
4 discusses transfer events in certain foodborne bacteria.

5
6 The genetic pool consists of a vast array of mobile genetic elements, which in theory
7 can be spread from species to species as every organism has the potential to take up
8 DNA. In essence the likelihood of an organism adopting extraneous DNA is limited
9 by a number of factors: the ability of the organism to take up the DNA, the readiness
10 to deliver DNA and the chance of the organisms being physically close to each other
11 at the same time in the same environment. Every organism that shares a particular
12 ecological niche or organisms passing through can be thought of as potential donors
13 and recipients (Hanssen and Ericson Sollid, 2006).

14
15 There are other barriers to horizontal gene transfer which may be significant. Plasmids
16 with the same incompatibility groups cannot survive in the same cell (Novick, 1987);
17 this is a huge consideration to the successful uptake of a conjugative plasmid. A good
18 deal of lateral gene transfer is dependent on bacteriophage and generalized
19 transduction, but the incidence and dissemination of these phages are not known
20 (Lindsay and Holden, 2006). The genetic background of the recipient, and its ability
21 to utilise the incoming genetic sequences have a huge impact on the success of a
22 transfer.

23
24 It has been shown in recent literature, that virulence genes have been transferred
25 between the same strains and sometimes between different strains of the foodborne

1 pathogens discussed in this review. *E. coli* has transferred genetic material to a variety
2 of bacteria including: *Klebsiella*, *Pseudomonas*, *Enterobacter*, *Salmonella* and other
3 *Enterobacteriaceae* (Schmidt et al., 1993, Paton and Paton, 1996), as well as
4 transferring genes to other *E. coli* strains, via phages and conjugative avenues
5 (Schwalbe et al. 1990). *L. monocytogenes* has transferred resistance determinants to
6 *Enterococci* and other *Listeria* strains via conjugation (Bertrand et al. 2005, Zhang et
7 al., 2007). Horizontal transfer of resistance determinants in *S. aureus* has not yet been
8 proven (Hanssen and Ericson Sollid, 2006), but the transfer of genes for the
9 preformed toxins which cause foodborne gastroenteritis has been shown to be linked
10 to transducing phages (Novick et al. 2001, Novick, 2003). The *Salmonella* genomic
11 island has been transferred between different *Salmonella enterica* strains and to *E.*
12 *coli* via conjugation (Doublet et al. 2005), and virulence determinants that lie on
13 resident prophages have been shown to transfer between different *Salmonella* serovars
14 and between the different phage families which resides on the *Salmonella* genome
15 (Mmolawa et al., 2002).

16

17 The foodborne bacterial pathogens discussed have transferred different genes to other
18 bacterial strains via conjugation, transformation and transduction. Transfer events
19 recorded have required that the donor and recipient organisms be present in the same
20 general environment, e.g. transfer of non-conjugative plasmids between mixed *E. coli*
21 strains in a biofilm (Maeda et al., 2006). So, recipients of virulence factors and
22 potential emerging pathogens from *E. coli*, *L. monocytogenes*, *S. aureus* and
23 *Salmonella* are bacteria such as commensals that are already share an environment
24 like the gastrointestinal tract with these organisms (Farthing, 2004). Future studies are

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- 1 needed to elucidate the mechanism of pathogen evolution in relation to foodstuffs, and
- 2 what part non-pathogenic organisms, like commensals play in this evolution.

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1 **Tables**

2 ***Table 1. Examples of transfer mechanisms evident in four foodborne***
 3 ***pathogens***

Organism	Mechanism	Example	Reference
<i>E. coli</i>	Conjugation	Plasmid transfer between different <i>E. coli</i> strains Plasmid transfer between different species	Johnson et al., 1994 Zhao et al., 2001
	Transduction	Stx-2 conversion of non-Stx-2 <i>E. coli</i>	Muniesa and Jofre, 2004
	Transformation	Non-conjugative plasmid transfer in biofilms	Maeda et al., 2006
<i>L. monocytogenes</i>	Conjugation	Plasmid transfer between <i>Listeria</i> strains Plasmid transfer to <i>Enterococcus faecalis</i>	Charpentier and Courvalin, 1999 Zhang et al., 2007
	Transduction	Generalised transduction	Hodgson et al., 2000
<i>S. aureus</i>	Transduction related	Transfer of SapIbov1	Úbeda et al., 2003
<i>Salmonella</i>	Conjugation	SGI-transfer to other <i>Salmonella</i> and <i>E. coli</i>	Doublet et al., 1995
	Transduction	Lysogeny with <i>Gifsy-1</i> , <i>Gifsy-2</i> and <i>Gifsy-3</i> in serovars other than <i>S. Typhimurium</i>	Figuroa-Bossi et al., 2001

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