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Gene transfer events and their occurrence

3

in selected environments

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15 *Abbreviations:* DNA, deoxyribonucleic acid; DNases, deoxyribonucleases; FDA,

16 Food and Drug Administration; GI, gastrointestinal tract; GM, genetically modified;

17 HC, haemorrhagic colitis HGT, horizontal gene transfer; HUS, haemolytic-uremic

18 syndrome ROAR, reservoirs of antibiotic resistance; STEC, shiga toxin-producing *E.*

19 *coli*; Stx, shiga toxin;

20 T-DNA, transferred DNA; U. S., United States; UHT, ultra heat treated; WHO, World

21 Health Organisation;

1 **Abstract**

2 Genes encoding virulence determinants are transferred between species in many
3 different environments. In this review we describe gene transfer events to and from
4 different species of bacteria, from bacteria to plants, and from plants to bacteria.
5 Examples of the setting for these transfer events include: the GI tract, the rumen, the
6 oral cavity, and in food matrixes. As a case study, the flux of virulence factors from
7 *E.coli* O157:H7 is described as an example of gene flow in the environment.

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1 **Introduction**

2 The movement of genes amongst different species occurs in nature, between closely
3 related organisms via the typical avenues of reproduction, and between different
4 species via horizontal gene transfer (HGT). An important consideration about the
5 concept of HGT is not whether genes can move into other organisms but whether
6 these genes confer enough of an advantage to make keeping them worthwhile. Gene
7 transfer events in nature have been assessed by three different approaches:
8 examination of the nucleotide sequences of similar genes in different organisms;
9 experimental demonstration of gene transfer under laboratory conditions and the
10 analysis of horizontal transfer events in microcosms and field surveys. These transfer
11 events have been shown to take place between different bacterial species; between
12 plants and bacteria; and between animals and plants (Droge et al., 1998). Some of the
13 evidence relating to these transfer events will be presented below. Gene transfer in
14 some selected environments will also be described in greater detail. The movement of
15 virulence factors from *Escherichia coli* O157:H7 will be presented as an example of
16 gene flux in the environment.

17

18 ***Transfer of genes between bacterial species***

19 The first documented evidence of *in vitro* transfer between Gram-negative bacteria
20 and Gram-positive bacteria was in 1987, when Trieu-Cuot et al. transferred the
21 plasmid pAT187 from Gram-negative *E. coli* to the Gram-positive strains:
22 *Enterococcus faecalis*, *Streptococcus lactis*, *Streptococcus agalactiae*, *Bacillus*
23 *thuringiensis*, *Listeria monocytogenes* and *Staphylococcus aureus* (Trieu-Cuot et al.,
24 1987). These transfer events lead to the belief that there could be “inter-Gram”
25 genetic exchange in natural conditions. In a review by Courvalin, it was proposed that

1 in nature, there is a bias in the gene flux from Gram-positive cocci to Gram-negative
2 bacteria, because of the barriers to heterologous gene expression that impedes the
3 expression of Gram-positive genes in Gram-negative bacteria and not vice versa
4 (Courvalin, 1994). The ubiquitous transfer mechanism between Gram-positive and
5 Gram-negative bacteria is thought to be conjugation due to the broad host range of
6 transfer and autonomous replication, however, the possibility of transfer via
7 transduction or transformation has not been ruled out between the two Gram type
8 strains. Lactic acid bacteria, including members of the genus *Enterococcus*,
9 *Lactobacillus* and *Lactococcus* have been shown to freely participate in the
10 communications superhighway that is the lateral transfer of genes, particularly
11 antibiotic resistance determining genes. Therefore lactic acid bacteria, like other
12 bacteria, participate in the transfer of antibiotic resistance characteristics across
13 species and genus borders. Identical genes responsible for antibiotic resistance are
14 found in commensal lactic acid bacteria, pathogenic bacteria and opportunistic
15 pathogenic bacteria. This has led to the suggestion that there are no barriers between
16 these three bacterial groups (Mathur and Singh, 2005).

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18 ***Transfer of genes from bacteria to plants***

19 *Agrobacterium tumefaciens* is a ubiquitous soil bacterium which can transfer DNA to
20 plants in genetic modification studies. This bacterium is responsible for crown gall
21 disease, which induces galls, or tumours, on certain plants. Gall formation is
22 attributable to the integration of bacterial transferred DNA (T-DNA) into the plant
23 chromosome. The advent of biotechnological techniques has enabled the manipulation
24 of T-DNA so as to facilitate the transfer of foreign genes into a wide variety of plants
25 (Broothaerts et al., 2005). *A. tumefaciens* was considered to be the only bacterial

1 species capable of this transfer, but Broothaerts group managed to obtain various plant
2 transformants using non-*Agrobacterium* strains *Rhizobium* sp., *Sinorhizobium meliloti*
3 and *Mesorhizobium loti*, albeit at a lower frequency rate than with *A. tumefaciens*.

4

5 ***Transfer of genes from plants to bacteria***

6 Genes from transgenic tobacco plants, with transgenes residing on the chloroplast
7 genome, have been shown to be transferred to *Acinetobacter*. In this case the bacterial
8 species housed a plasmid that contained homologous sequences to the chloroplast
9 genome (Kay et al., 2002). Despite the fact that plant nucleases, microbial nucleases
10 and shear forces contribute to the destruction of plant DNA, intact DNA sections can
11 actually be located in the environment. DNA found in the environment can be
12 protected by adsorption to sand and clay particles, making it more resistant to the
13 action of DNases (Davison, 1999, Gay, 2005). Gene transfer between GM plants and
14 bacteria has occurred in a soil environment, where a prerequisite for this transfer was
15 a homology between donor and recipient DNA (Eede et al., 2004). Many GM plants
16 have been developed with antibiotic genes that retain their original bacterial promoter.
17 This is because the gene of interest was originally engineered into an *E. coli* cloning
18 vector containing the antibiotic resistant genes. *Agrobacterium* binary vector systems
19 also sometimes contain bacterial antibiotic resistance genes and have been used to
20 introduce transgenes into plants (Gasson, 2000).

21

22 Currently, many GM plants are cultivated for different reasons ranging from: carriage
23 of genes resistant to a particular pesticide, herbicide or insect; the rendering of plants
24 male-sterile; delaying the ripening of fruit; or simply to be a transformation marker
25 (Ishimania et. al., 2006; Uzogara, 2000). These transgenes in GM plants often contain

1 prokaryotic sequences. There has been major concern into the potential for these
2 transgenes to migrate from GM plants to human or animal intestinal microbes
3 (Heritage, 2004; Heritage, 2005). One of the main concerns is that antibiotic
4 resistance genes used in the construction of GM plants may end up in pathogenic
5 bacteria that reside in the gastrointestinal (GI) tract (Wilcks et al., 2004).

6

7 Many species of bacteria may develop natural competence, or the ability to take up
8 naked DNA, in the gastrointestinal (GI) tract, and this development, along with
9 factors like the integrity and quality of the DNA from GM plants that reaches the GI
10 tract, are major factors when considering the risk associated with consumption of GM
11 foods. The GI tract is broken up into many parts, with the proximal portion being the
12 part where the DNA is released from the food matrix and is exposed to breakdown via
13 nuclease activity and low pH in the stomach (Wilcks et al., 2004). Many of the
14 antibiotic resistance genes present in GM plants are under the control of plant
15 promoters and would normally need the addition of a bacterial promoter in order to be
16 functional in a recipient bacterial cell. However, it was also shown that the sequences
17 of the plant promoter themselves were recognised by the bacterial transcription
18 apparatus (Jacob *et al.*, 2002, Lewin *et al.*, 1998). Duggan et al. found that free DNA
19 survives in a functional state for a considerable amount of time in ovine saliva but
20 survives a much shorter amount of time in rumen fluid and effluent due to high
21 concentrations of free endo and exo-nucleases (Duggan et al., 2000), indicating that
22 the possibility for natural transformation in the oral cavity is a distinct possibility.

23

24 Recently, Kleter et al. (2005) evaluated the health considerations regarding the
25 transfer of microbial transgenes present in genetically modified crops. 10 different

1 transgenes routinely used in market-approved crops were examined using a number of
2 criteria e.g. microbial source, natural function of gene examined, prevalence of gene
3 in other organisms. Two of the transgenes examined were DNA adenine methylase
4 (*dam*) and the β -glucuronidase (*uidA*) genes from *E. coli* and the *cry* genes from
5 *Bacillus thuringiensis*. The microbial genes reviewed in the study did not give rise to
6 any health concerns, but Kleter et al. advised that any transgenes not mentioned in this
7 study should be subjected to examination using their outlined criteria.

8

9 A review by Thomson (2001) outlines the steps and the consequences of gene transfer
10 from a GM crop to intestinal bacteria. The author examines the various worst-case
11 scenarios (where gene transfer is affected) and the consequences of each step are
12 discussed. It was found that horizontal gene transfer events between GM crops and
13 intestinal bacteria have occurred, but they are quite rare. However, because these rare
14 events may have an ecological impact, genes introduced into a GM plant should be
15 subjected to risk assessment.

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17 In a review by Goldstein et al. (2005), where they discussed human safety
18 considerations of genetically modified plants with respect to antibiotic resistance
19 genes, the authors came to the conclusion that at the moment there is no
20 documentation of intact functional antibiotic resistance gene transfer from plants to
21 bacteria, and because these genes are readily available in bacterial reservoirs already,
22 the frequency of such plant to bacteria transfer events is trifling. The selective effect
23 of antibiotic use in the environment contributes more to the evolution of resistant
24 strains by spontaneous mutation and transfer of existent resistant plasmids.

25

1 A number of years ago, both the U. S. Food and Drug Administration (FDA) and the
2 World Health Organisation (WHO) concluded that there is no risk in consuming DNA
3 from biotech crops. (WHO, 1991; US-FDA, 1992). The reasoning behind their
4 conclusion is that humans and other animals have always consumed DNA from a
5 wide variety of sources including plants, animals, bacteria, parasites and viruses, so
6 consuming DNA from another source, such as biotech crops, should cause no extra
7 risk.

8

9 **Gene transfer in selected environments**

10 Gene transfer events occur in many diverse environments. The most significant
11 environment for food borne pathogens is the gastrointestinal tract, with the possibility
12 of commensal bacteria acting as a reservoir for the spread of virulence determinants
13 from transient microbiota (Farthing, 2004). Horizontal gene transfer events have also
14 been demonstrated in the rumen (McCuddin et al., 2006), in foodstuffs (Brautigam et
15 al., 1997), in biofilms present on food processing equipment (Maeda et al., 2006) and
16 in the oral cavity (Mercer et al., 1999) and are discussed below.

17

18 ***Transfer of genes in the gastrointestinal tract***

19 The gastrointestinal tract (the alimentary canal or the gut), is the system of organs
20 within multicellular animals which takes in food, digests it to extract energy and
21 nutrients, and expels the remaining waste. More than 500 species of bacteria colonize
22 the gastrointestinal (GI) tract (Gilmore and Ferretti, 2003). Differences in the type and
23 total numbers of bacteria present depend, for example on the spatial location in the GI
24 tract (Farthing, 2004). Microbial flora of the gut form part of a dense population

1 existing in close proximity, which often are part of biofilms. This environment is an
2 ideal one for genetic transfer between different bacterial types (Scott, 2002).

3

4 There are numerous examples of conjugation as a means of gene transfer with regards
5 food and the intestine (Eede et al., 2004), therefore it can be thought of as a common
6 and very efficient way to transfer genes to these environments. The digestive tract of
7 animals is thought to be conducive to gene transfer between lactococci and enterics,
8 as supported by genome sequence analysis (Bolotin et al., 2004). Maisonneuve et al.
9 (2000) investigated whether yoghurt had positive effects on plasmid transfer and
10 transconjugant survival in the digestive tract of mice associated with human faecal
11 flora, and found that the rates of transfer were at a lower efficiency and there was no
12 stimulating effect. Salyers et al. (2004) have recently proposed that the human
13 intestine, (i.e.) the GI tract, is rife with gene transfer events, with relatively harmless
14 bacteria casually swapping genes between themselves. This becomes a problem if
15 bacteria that normally are transitory in the human colon acquire virulence genes,
16 particularly resistance genes, by conjugation, thus converting a non-pathogen into a
17 potential pathogen via the transfer of virulence genes. Effectively, these microbial
18 intestine dwellers act as vectors for antibiotic resistance genes (Salyers et al., 2004).
19 Tetracycline and erythromycin resistance determinants encoded on transposons were
20 shown to be transferable from *Enterococcus faecalis* to *E. coli* and *L. monocytogenes*
21 in the digestive tract of mice (Doucet-Populaire et al., 1991, Doucet-Populaire, 1992).

22

23 Netherwood et al. (1999), performed *in vitro* and *in vivo* studies on the transfer of
24 genes from a genetically modified probiotic in the avian GI tract, and came to the
25 conclusion that the *in vivo* rate of transfer is higher than the rates obtained *in vitro*,

1 and that the true rate of transfer was underestimated due to the asynchronous nature of
2 bacterial cells in the GI tract. Anaerobic bacteria make up 99% of the human gut flora
3 (Vedantam and Hecht, 2003).

4

5 Commensal organisms, present in the gastrointestinal tract are thought to be reservoirs
6 of determinants such as antibiotic resistance. There are a number of initiatives (e.g.
7 reservoirs of antibiotic resistance (ROAR); dedicated to determine the dissemination
8 levels of non-pathogenic antibiotic resistance) which try to elucidate the importance
9 of non-pathogens as carriers of virulence determinants. Commensal bacteria can act as
10 a reservoir for the dissemination of virulence genes from transient bacteria. Bacteria
11 ingested with food are transient inhabitants of the GI tract; therefore they can
12 contribute to the gene pool available to resident commensal microflora for genetic
13 exchange. Studies by Blake et al. (2003) using simulated porcine ileal conditions
14 provided clear evidence that antibiotic resistance determinants could be transmitted
15 between commensal and pathogenic members of the Enterobacteriaceae.

16

17 The evidence for gene transfer in the colon has been observed in *Bacteriodes* species
18 by Shoemaker et al. (2001), where resistance determinants from other Gram-negative
19 and also Gram-positive bacteria were found as part of the genome as conjugative
20 transposons. Frequently, the environment of the gut is exposed to low levels of
21 antibiotics (therapeutic agents, growth promoters, residues from food), which have
22 been shown to stimulate the transfer of mobile genetic elements, such as conjugative
23 transposons (Scott, 2002) and prophage elements (Úbeda et al., 2005). The murine GI
24 tract has proven to be an environment where H-19B, a shiga toxin 1(Stx1)-encoding
25 phage could be transmitted between two *E. coli* strains and the production of

1 infectious virions which are capable of infecting other *E. coli* strains in the GI tract
2 (Acheson et al., 1998).

3

4 ***Transfer of genes in the rumen***

5 The rumen is the first compartment of cattle, sheep and goats. Without the rumen,
6 these animals would not be able to digest high fibre plant materials as all of the
7 digestion that takes place in the rumen is due to the presence of a mixture of micro-
8 organisms (Weimer, 1992). These microbes represent a diverse mixture of prokaryotic
9 and eukaryotic organisms (McCuddin et al., 2006). With this mixture of micro-
10 organisms, the opportunity for gene exchange in the rumen environment is great.
11 Transfer of antibiotic resistance determinants in the rumen was first documented in
12 sheep in the 1970s, and since then indirect evidence has mounted for rumen transfer
13 events. Rumen protozoa have been shown to play a part in gene transfer between
14 bacteria inhabiting the rumen. McCuddin et al. (2006) investigated this role further
15 between an antibiotic-resistant *Klebsiella* donor and an antibiotic-resistant *Salmonella*
16 recipient, and found that inhabiting rumen flora did indeed enhance gene transfer of
17 antibiotic resistance between these bacterial species.

18

19 ***Transfer of genes in food***

20 Food matrices such as ultra heat treated (UHT) milk, cacao drink and tomato juice
21 have been reported to support transformation when external DNA was added along
22 with the bacterial strains. Transfer of DNA to bacteria in foods by transformation
23 necessitates unbroken DNA molecules of a certain length and a certain amount of
24 homology between the bacterial DNA and the extraneous DNA present in the food
25 (Kharazmi et al., 2003). It is also necessary that the recipient bacteria are

1 transformable in the food environment. Natural transformation in complex food
2 matrices was examined in a naturally competent bacterium, *B. subtilis*, by Brautigam
3 et al. (1997). A variety of milk products: UHT milk with different fat levels;
4 pasteurised milk; and chocolate milk were evaluated as environments for
5 transformation. Competence development occurred in all the milk products tested.
6 Transformation of *E. coli* in a variety of foodstuffs was investigated and it was found
7 that the highest transformation frequencies occurred in milk, soy drink, tomato and
8 orange juice, and that DNA was released and taken up by *E. coli* under food
9 processing conditions, meaning that gene transfer can occur in these environments,
10 which is a food safety concern (Bauer et al., 1999). Gabin-Gauthier et al., (1991)
11 found that conjugal transfer between lactococci occurred during cheese making, but at
12 a lower frequency than under laboratory conditions.

13

14 Biofilms, biologically active matrices which consist of cells and extracellular
15 substances, have become an important issue with regards to food hygiene. Biofilms
16 are formed on any submerged surface in any environment where bacteria are present
17 and can form on food products or food product contact surfaces, such as pipes and
18 rubber seals, leading to undesirable potential contamination of the foodstuff.
19 Attachment of bacterial biofilms to foodstuffs or contact surfaces leads to problems
20 with hygiene and economic losses due to spoilage of food. Many pathogens have been
21 shown to persist in biofilms, also making these biological matrices a food safety
22 problem. Getting rid of bacteria which are present in biofilms raises many problems.
23 Bacteria present in biofilms exhibit increased resistance to antimicrobial agents,
24 probably due to many factors including: reduced diffusion of the agent; reduction of
25 bacterial growth rate and the production of degradative enzymes. Bacteria in biofilms

1 have also been shown to have decreased susceptibility to a wide variety of antibiotics
2 (Kumar and Anand, 1998). *E. coli*, which is not normally considered as naturally
3 transformable requiring exposure to high Ca^{2+} concentrations to develop competence,
4 has been shown to express modest competence within a colony biofilm (Maeda et al.,
5 2004). Recently, horizontal transfer of nonconjugative plasmids in a colony biofilm of
6 *E. coli* has also been shown (Maeda et al., 2006). The transfer of conjugative plasmids
7 in biofilms has been discussed by Molin et al. (2003) and it seems that there is a
8 difference between conjugative transfer of a R1 plasmid between suspended and
9 sessile (biofilm-inhabiting) *E. coli* pairs. Transconjugants appeared very rapidly and
10 their numbers increased at a high rate in the biofilm situation, whereas when the cells
11 were suspended in a chemostat, transconjugants appeared more gradually. The
12 incidence of antibiotic-resistant enterobacteria was investigated in a variety of
13 agricultural foodstuffs by Boehme et al. (2004), who found that the amount of
14 coliform bacteria present on “common vegetables” (which included carrots,
15 cauliflower, mushrooms, lettuce) was a few orders in magnitude lower than the
16 coliform bacteria present on sprouts, which were highly contaminated. Antibiotic
17 resistance was rife on the sprout-contaminating microflora, whereas only a few
18 resistant strains were found in relation to the common vegetable coliforms.

19

20 Wang et al. (2006) examined retail food samples (ranging from milk, cheeses,
21 yogurts, shrimp, deli beef, deli turkey, mushroom and spinach) for the presence of
22 antibiotic resistance determinants. In the majority of retail food examined, antibiotic
23 resistant micro-organisms were detected, indicating that the prevalence of antibiotic
24 resistant commensal organisms in foodstuffs is quite high.

25

1 ***Natural genetic transformation in the oral cavity***

2 The fate of free DNA in saliva was investigated by Mercer *et al.* (1999), and was
3 found to be able to survive for a considerable amount of time in a semi-degraded
4 state. This study also found that this DNA was able to transform naturally competent
5 *Streptococcus gordonii* DL1, a natural member of the oral microflora.

6

7 **The flux of virulence factors of *E. coli* O157:H7**

8 Virulence genes of *E. coli* are present on several mobile genetic elements such as
9 plasmids, phages, transposons and pathogenicity islands. New *E. coli* pathotypes, with
10 new combinations of genetic information are constantly emerging. *E. coli* represents a
11 potential pool of virulence genes which may play a key role in the origin of emerging
12 diseases caused by *E. coli* and other bacteria, and which may occur in yet unexplored
13 ecological niches (Kuhnert *et al.*, 2000). In this section, the flux of Shiga toxin-
14 producing *E. coli* (STEC) virulence factors in the bacterial population in the farm
15 environment and potential adjacent areas will be examined.

16

17 STEC have been isolated from many animal species, including sheep, goat, deer, pig,
18 cat, horse and gull, however cattle appear to be the major reservoir for *E. coli*
19 O157:H7 and other STEC (Renter and Sargeant, 2002). The animals harbour this
20 pathogen in their GI tracts and shed the bacteria in their faeces (Chapman *et al.*,
21 1993). Enteric bacteria, like *E. coli* are able to survive in different environments,
22 while the gut is their natural ecosystem (Witte, 2000). The ability of *E. coli* O157:H7
23 to survive under environmental conditions outside of the gastrointestinal tract is one
24 of the factors enabling gene transfer from this pathogen in the environment. Faeces
25 are an important vehicle for the distribution of *E. coli* O157, as this pathogen is able

1 to survive up to 18 weeks in this medium (Wang et al., 1996, Kudva et al., 1996).
2 Raw manure, untreated and treated slurry which all include faeces, is often utilized to
3 fertilize soil (Jones 1980; Mechie et al., 1997), which aids in the spread of this
4 pathogen. *E. coli* O157:H7 has been shown to survive in non-aerated ovine manure
5 pile for more than 1 year and in aerated ovine manure or bovine manure piles several
6 months (Kudva, 1998). The pathogen has survived in drinking water troughs
7 (Hancock et al. 1998; LeJeune et al., 2001) for at least 6 months and also in farm
8 water for long periods (McGee et al., 2002).

9
10 A great diversity of the *stx*-(shiga toxin gene) and *eae*-(intimin gene) positive *E. coli*
11 isolates were detected in slaughterhouse wastewater that was ready to be released into
12 the environment (Loukiadis et al., 2002). River water collected upstream from the
13 slaughterhouses show a lesser contamination with this pathogen than river water
14 collected downstream from the slaughterhouses (Loukiadis et al., 2002). *E. coli*
15 O157:H7 has also been isolated, for example from animal feed and flies at dairy farms
16 (Shere et al., 1998). Barker et al. (1999) have shown that *E. coli* O157:H7 survives
17 and replicates in a common environmental protozoan, *Acanthamoeba polyphaga*,
18 which are widely distributed in soils and effluents. This ability to survival in protozoa
19 may have enabled the persistence of *E. coli* O157:H7 in the natural environment
20 (Brown *et al.*, 2002). High levels of transfer of conjugative and mobilizable plasmids
21 from *E. coli* to a wide variety of strains of *Proteobacteria* were observed in the gut of
22 the soil microarthropod *Folsomia candida* (Hoffmann et al., 1998). In another study, a
23 conjugative plasmid was transferred between strains of *E. coli* in the gut of *Rhabditis*
24 *nematodes* (Adamo and Gealt, 1996).

25

1 *E. coli* O157:H7 possesses a potent combination of virulence factors. The main
2 virulence factors in the disease progression of haemorrhagic colitis (HC) and
3 haemolytic-uremic syndrome (HUS), caused by this organism, are Shiga toxins (Stx1
4 and Stx2) which are encoded by bacteriophages (O'Brien et al., 1984). It has been
5 suggested by Urdahl et al. (2003) that animals kept in pens probably maintain a higher
6 level of Stx in the intestine than tethered animals, because they would have more
7 faecal-oral contact, thus more chance of cross-contamination. In Norwegian dairy
8 cattle herds it is thought that loose housing presents the major risk factor for
9 occurrence of Stx2 (Vold et al., 2000).

10

11 Transfer of shiga toxin between STEC and non STEC serotypes and STEC and other
12 members of the Enterobacteriaceae occurs via the transduction of *stx* phages (Beutin et
13 al., 1999; Schmidt et al., 1999). In several studies the intrainstestinal transmission of
14 *stx*-bearing bacteriophages to new bacterial hosts has been demonstrated, for example
15 *in vivo* (Acheson et al., 1998) and *in vitro* (Schmidt et al., 1999). Phages were also
16 shown to be transmitted extraintestinally (Muniesa and Jofre; 2004). Infectious Shiga
17 toxin phages have been observed in the sewage of various countries and in faecal
18 contaminated rivers (Muniesa et al., 1999; Muniesa et al., 2000; Muniesa and Jofre,
19 2004; Tanji et al., 2003), and also in surface water (Dumke et al., 2006). These phages
20 have been shown to exhibit higher persistence to natural inactivation and disinfection
21 treatments in aquatic environments (Muniesa et al., 1999; Tanji et al., 2003).
22 Naturally occurring phages that carry the Stx2 gene and infect *E. coli* O157:H7 are
23 able to persist in the water environment more successfully than their host bacteria and
24 show a higher resistance than their host bacteria to chlorination and heat treatment.
25 Furthermore, Stx2 genes which are incorporated in free phages persist outside the gut

1 much more successfully than the genes incorporated in the bacterial genome (Muniesa
2 et al., 1999). In several studies of cattle, seasonal fluctuations of faecal shedding of *E.*
3 *coli* 0157:H7 was observed. No significant seasonal differences in the levels of
4 shedding of Stx2 gene-carrying bacteria in monitored human wastewater treatment
5 plant and contaminated river waters was observed in a Japanese study (Kurokawa *et*
6 *al.*, 1999). Bacteriophages may be an important pool of toxigenic strains based on the
7 numbers and persistence of bacteriophages in natural environments (Muniesa *et al.*,
8 1999). García-Aljaro et al. (2004) indicated the potential contribution of
9 bacteriophages to the mobility of the Stx2 gene should not be underestimated.
10 Additionally the occurrence of the Stx2 gene in populations detected in sewage
11 (phages or bacteria) demonstrates an exchange of this gene between these populations
12 (García-Aljaro et al., 2004). The aminoglycoside apramycin has been used extensively
13 in livestock in the UK since 1978 (Yates et al., 2004), studies have shown that
14 apramycin-resistant commensal *E. coli* are absent in cattle that have never been
15 treated with aminoglycosides.

16

17 **Conclusion**

18 Horizontal gene transfer occurs in many different locations under many diverse
19 conditions. We have seen in this review that gene transfer events have occurred from
20 plants to bacteria; bacteria to plants; in the gastrointestinal tract; in the oral cavity; and
21 on food surfaces, to name a few instances. There is no doubt that there are still
22 numerous places and circumstances to be discovered where gene transfer events can
23 occur. Genetic exchange is widespread in the environment relevant to food borne
24 microflora. The three mechanisms of gene transfer, conjugation, transformation and

1 transduction, are represented in this environment, and conjugation seems to be the
2 most ubiquitous mechanism present from the studies examined.

3

4 We have seen from this review that natural transfer of genetic material between
5 bacteria and the environment is ubiquitous, and this transfer is necessary for the
6 generation of diverse species. Mobile genetic elements, by various methods, move in
7 and out of bacterial genomes adding extra abilities and allowing proliferation of
8 bacterial species in new niches. One of the main requirements for transfer of virulence
9 determinants between strains is the proximity of the donor and recipient organisms.

10 This information should be taken into consideration in future risk assessment of
11 foodborne bacterial pathogens.

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