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Review

Factors influencing horizontal gene transfer in the intestine

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Abstract

Antibiotic resistance (AR) is ancient. Use of antibiotics is a selective driving force that enriches AR genes and promotes the emergence of resistant pathogens. It also has been widely accepted that horizontal gene transfer (HGT) occurs everywhere and plays a critical role in the transmission of AR genes among bacteria. However, our understanding of HGT processes primarily build on extensive *in vitro* studies; to date, there is still a significant knowledge gap regarding *in situ* HGT events as well as the factors that influence HGT in different ecological niches. This review is focused on the HGT process in the intestinal tract, a 'melting pot' for gene exchange. Several factors that potentially influence *in vivo* HGT efficiency in the intestine are identified and summarized, which include SOS-inducing agents, stress hormones, microbiota and microbiota-derived factors. We highlight recent discoveries demonstrating that certain antibiotics, which are widely used in animal industry, can enhance HGT in the intestine by serving as DNA-damaging, SOS-inducing agents. Despite recent progress, research on *in vivo* HGT events is still in its infancy. A better understanding of the factors influencing HGT in the intestine is highly warranted for developing effective strategies to mitigate AR in animal production as well as in future agricultural ecosystems.

Keywords: horizontal gene transfer, antibiotic resistance, gut microbiota, SOS response, risk factors.

Introduction

Discovery and development of various antibiotics in the past century are a significant milestone and one of the most successful therapeutic strategies to treat bacterial infections in people and animals. However, growing antibiotic resistance (AR) has compromised the efficacy of antibiotics, posing a serious threat to animal health, food safety and public health. In particular, emergence of multidrug-resistant (MDR) bacteria, which usually contain resistance determinants in mobile elements (e.g. transposons and plasmids), have put a severe burden on animal industry and human society (Marshall and Levy, 2011; Szmolka and Nagy, 2013; Michael *et al.*, 2015). In the USA alone, infections caused by MDR organisms are estimated to cost $20 billion annually in direct health care costs, plus an additional $35 billion in costs due to lose of productivity (Centers for Disease Control and Prevention, 2013).

Bacteria have evolved multiple strategies to counteract bactericidal or bacteriostatic effects of antibiotics (Wise, 2002; Andersson and Hughes, 2010; Fisher *et al.*, 2017). In past decades, extensive efforts have been placed on the elucidation of the molecular basis of AR and development of innovative mitigation strategies, such as development of β-lactamase inhibitors (Harris *et al.*, 2015; Bush and Bradford, 2016). Recently, with the aid of high-throughput sequencing and metagenomic approaches, resistome studies have revealed a much higher level of AR diversity and novelty in different niches than previously anticipated (Pehrsson *et al.*, 2013; Harris *et al.*, 2015; Crofts *et al.*, 2017). In addition, extensive *in vitro* studies also have indicated that the process of horizontal gene transfer (HGT) contributes to the exchange of AR genes between bacterial organisms (same or different species) in different ecological niches, consequently playing a critical role in the dissemination and evolution of AR genes in bacteria (Broaders *et al.*, 2013; Huddleston,

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2014). However, to date, there is still a significant knowledge gap regarding *in situ* HGT events, as well as the factors influencing HGT in different environments (e.g. the intestine), which has impeded development of effective strategies to mitigate AR across the food chain.

In this review, we provide a brief overview of HGT in bacteria, as well as the uniqueness of the intestinal tract for efficient HGT to occur among bacterial organisms. We also identify and summarize recent progress on the factors potentially promoting *in vivo* HGT in the intestine. In particular, we highlight recent mechanistic studies demonstrating that certain antibiotics, such as fluoroquinolones (FQs) and β-lactams, can enhance HGT in the intestine by serving as DNA-damaging, SOS-inducing agents.

Horizontal gene transfer

The HGT process enables exchange of genetic material between bacterial cells and plays a critical role in bacterial evolution and adaptation to their environment (Ochman *et al.*, 2000). As a result, bacteria, even within the same species, could exhibit significant plasticity in genome for successful survival in different ecological niches. It has been widely accepted that there are three major forms of HGT: conjugation, natural transformation, and transduction (Ochman *et al.*, 2000). Almost all DNA sequences, including AR and virulence genes, could be transferred between bacterial cells through HGT (Davies, 1996; Davies and Davies, 2010). The major features and recent advances of the three types of HGT are briefly summarized below.

Conjugation

Conjugation is a stepwise DNA transfer process through a complex type IV secretion system (Curtiss, 1969; Alvarez-Martinez and Christie, 2009). Conjugation needs intimate cell-to-cell contact for bridge formation between the mating pair via a conjugative pilus that belongs to type IV secretion system. Besides plasmids, some specific large mobile elements in the chromosome, such as integrating conjugative elements (ICEs) (Wozniak and Waldor, 2010), can be excised from chromosomes and subsequently transferred through the conjugative apparatus (Burrus *et al.*, 2002; Burrus and Waldor, 2004). For example, SXT, a large ICE (~100 kb), and its closely related ICEs were not found prevalent among most *Vibrio cholerae* O1 and O139 clinical isolates until the early 1990s (Amita *et al.*, 2003). SXT usually bears multiple cassettes conferring resistance to chloramphenicol, sulfamethoxazole, and trimethoprim. SXT was initially found in *V. cholerae* O139 serogroup in late 1992 on the Indian subcontinent, and spread to most O1 and O139 clinical isolates in Asia (Amita *et al.*, 2003), most likely via conjugation (Waldor *et al.*, 1996). More in-depth information of conjugation is available in several excellent reviews (Curtiss, 1969; Smith *et al.*, 1981; Smith, 1991; Christie and Vogel, 2000; Christie *et al.*, 2005; Alvarez-Martinez and Christie, 2009).

Natural transformation

Natural transformation is a phenomenon through which bacterial cells can directly take up extracellular DNA (either linear fragment or circular plasmid), and subsequently maintain them. Since natural transformation was first discovered in *Streptococcus pneumoniae* in 1928 (Griffith, 1928), over 80 species with high-level natural transformation ability have been identified, which include a panel of gastrointestinal pathogens, such as *Campylobacter jejuni*, *Campylobacter coli*, *Helicobacter pylori*, and *V. cholerae* (Lorenz and Wackernagel, 1994; Johnsborg *et al.*, 2007; Johnston *et al.*, 2014). However, many enteric bacterial species do not display natural transformation ability under laboratory conditions. This fact, in combination with the well-known information regarding destruction of foreign DNA via restriction-modification systems in recipient cells (Palmer and Marinus, 1994), raises a significant question: Is natural transformation a common and frequent HGT event in the intestine? It is likely that some bacterial organisms may conditionally acquire high-level natural transformation capability in the intestine at a specific growth stage or in response to specific cues *in vivo*. This speculation is partly supported by recent work showing that the commensal, *Escherichia coli*, previously not considered as a naturally competent bacterium, could display increased natural transformation ability upon static cultivation after the stationary phase at 37 °C (Sun *et al.*, 2006). To better understand the role of natural transformation in HGT in the intestine, examination of specific conditions or cues to trigger bacterial competence for DNA uptake is highly warranted in the future.

Transduction

Transduction is mediated by bacteriophages, viruses with specificity to bacterial hosts. Bacteriophages undergo the life cycle between integration into bacterial genome (prophages) and lytic growth stage. During lytic growth stage, a bacteriophage may be incorrectly excised from its host genome, leading to packaging of some host genetic material (donor DNA) into newly synthesized viral particles, which subsequently transfer donor DNA into another bacterial cell (recipient) through infection. A metagenomic investigation suggested that all functional bacterial genes were distributed in up to 50–60% of bacteriophages (Dinsdale *et al.*, 2008); therefore, the bacteriophages in the gut collectively form a huge gene reservoir and are expected to play a significant role in HGT among intestinal bacteria.

The intestinal tract: a melting pot for HGT

The intestinal tract is a complex ecosystem containing all elements for efficient HGT, which enables bacteria to exchange genetic materials, including but not limited to AR and virulence genes, for adaptation to hostile conditions in the intestine (Capozzi and Spano, 2009). Animal gut is increasingly recognized as a 'melting pot' for exchange of genetic materials across

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various phylogenetic distances (Shterzer and Mizrahi, 2015). A recent comparative microbiome study supported cross-species HGT in the gut. Genes encoding carbohydrate-active enzymes (porphyranases and agarases), originally identified in the marine bacterium, *Zobellia galactanivorans*, were observed to be transferred to the gut bacterium, *Bacteroides plebeius*, in Japanese but not in North American individuals (Hehemann *et al.*, 2010; Sonnenburg, 2010). This interesting observation may be explained by the extensive ingestion of *Z. galactanivorans*-containing seaweed, e.g. sushi, in the Japanese diet (Hehemann *et al.*, 2010; Sonnenburg, 2010). Another example of a genetic material melting pot via HGT in the intestinal tract is based on genomic examination of the gut archaeon, *Methanobrevibacter smithii*. Approximately 15% of genes in the genome of *M. smithii* were speculated to be acquired from co-resident gut bacteria, as evidenced by their GC content as well as adjacent location to mobile genetic elements (Lurie-Weinberger *et al.*, 2012). Following are several unique features making the intestinal tract a perfect melting pot for HGT.

First, microbial load in the gastrointestinal tract is enormously high, which creates an optimal environment for active microbial interaction. It is estimated that the human gastrointestinal tract is inhabited by more than 1000 bacterial species and 100 billion bacterial cells, which is about ten times the amount of total human cells (Ley *et al.*, 2006). The number of genes in the human gastrointestinal microbiome is more than 100 times that of the human genome (Ley *et al.*, 2006). The bacterial density in the gastrointestinal tracts of various food animals is also as high as 10^{10}– 10^{11} cells ml^{-1} (Whitman *et al.*, 1998).

Second, the gastrointestinal tract is a hostile environment with multiple levels of stress for intestinal bacteria. These stresses include but are not limited to pH, bacterial and host metabolites (e.g. bile salts), host defense factors (e.g. antimicrobial peptides), nutritional immunity (e.g. iron limitation), respiratory oxygen species, limited oxygen level, and bacterial competition (Kortman *et al.*, 2014). Clearly, the antibiotics via oral administration can exert both transient and long-lasting stress to the gastrointestinal bacteria. Some of these stress signals, regardless of whether they are indigenous or exogenous, may induce and promote an *in situ* HGT process, which will be comprehensively reviewed in a separate section hereinafter.

Third, the gastrointestinal tract serves as an immense reservoir for AR genes which can be acquired by other gastrointestinal bacteria via HGT (Salyers *et al.*, 2004). According to the Antibiotic Resistance Genes Database (ARDB, http://ardb.cbcb.umd.edu/), there are 23,137 known AR genes against 249 different antibiotics (Liu and Pop, 2009). A metagenome study identified 1093 AR genes from the gastrointestinal samples of 162 individuals (Sommer *et al.*, 2009). A recent metagenomic analysis of gastrointestinal microbiomes from 275 individuals revealed AR genes conferring resistance to 53 antibiotics (Ghosh *et al.*, 2013). This study also found that multiple AR genes are clustered and linked to integrase and transposase, suggesting the AR genes are part of mobile genetic elements (Ghosh *et al.*, 2013). In food animals, the intestinal microbiome also serves as an immense reservoir for AR genes. In a metagenomic study using intestinal samples from conventionally raised beef cattle with no exposure to therapeutic antibiotics, approximately 3.7% of the sequences encoded AR genes to antibiotic and toxic compounds (Durso *et al.*, 2011). In a large-scale swine study, high-capacity quantitative PCR arrays detected 149 unique AR genes among all swine fecal samples tested (Zhu *et al.*, 2013).

Notably, to date, our understanding of HGT in the gut primarily build on extensive *in vitro* studies. It is still largely unknown how HGT occurs in the intestine, particularly in terms of the *in vivo* factors influencing HGT efficiency in the intestine. The limited *in vivo* studies using rodent models only provided evidence of plasmid-mediated HGT transfer of AR genes, with a very narrow scope of HGT events in the gut (Schlundt *et al.*, 1994; Feld *et al.*, 2008; Garcia-Quintanilla *et al.*, 2008). Thus, research on *in vivo* HGT is still in its infancy. A better understanding of the factors influencing HGT in the intestine would help to develop practical and innovative strategies to reduce the threat and risk of AR in animal production as well as in agricultural ecosystems.

Factors potentially influencing HGT processes in the intestine

The efficiency of HGT can be influenced by various environmental factors, which have been identified and characterized by recent mechanistic, molecular, and microbiological studies. Despite the *in vitro* nature of these studies, many factors could be present in the intestine and potentially influence HGT efficiency *in vivo*. Thus, in this section, we comprehensively review and discuss the factors potentially influencing HGT processes in the intestine.

SOS response and SOS-inducing antibiotics

The SOS response, regulated by *lexA* and *recA* genes, is a global stress response triggered by DNA damage, in which DNA repair and mutagenesis are induced (Erill *et al.*, 2007). The transcription of SOS response genes is normally repressed. Upon chromosomal damage, the exposed single-stranded DNA can attract RecA to form nucleoprotein filaments, which in turn activates the expression of SOS genes by facilitating the cleavage of the LexA repressor (Beaber *et al.*, 2004; Schlacher and Goodman, 2007). Activation of SOS response by UV irradiation or DNA damaging agents can influence multiple aspects of cellular functions including DNA repair-mediated, mutation-based AR development (Radman, 1975; Matic *et al.*, 1995). The findings from recent extensive studies further provided compelling evidence that induction of the SOS response by other agents could promote HGT in bacteria. In particular, a panel of antibiotics, such as FQs and β-lactams, can serve as DNA-damaging agents to promote HGT of virulence as well as AR genes in bacteria.

Using conjugation and gene expression assays, Beaber *et al.* (2004) demonstrated that induction of the SOS response using a SOS-inducing agent, mitomycin C, and ciprofloxacin, a FQ

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antibiotic, markedly enhanced ICE transfer (>300-fold) in both *E. coli* and *V. cholerae*. Mechanistic work showed that the SOS response increased the expression of genes required for ICE transfer by inactivating the repressor, SetR, consequently enhancing HGT frequency (Beaber *et al.*, 2004).

The FQs and β-lactams can also serve as SOS-inducing agents to trigger competence and enhance natural transformation ability of bacterial cells (Charpentier *et al.*, 2012). For example, FQ antibiotics, which inhibit DNA gyrase, can break the DNA double strand, consequently inducing competence and enhancing the transformation in *S. pneumoniae* (Prudhomme *et al.*, 2006).

Recently, increasing evidence also indicated that SOS-inducing agents, particularly the SOS-inducing antibiotics, can trigger bacterial prophage induction, consequently enhancing transduction frequency (Comeau *et al.*, 2007; Allen *et al.*, 2011). For example, upon ciprofloxacin treatment, the genes associated with the SOS response as well as those for a viable bacteriophage were induced in *Burkholderia thailandensis* (Ulrich *et al.*, 2013). Modi *et al.* (2013) evaluated the effect of oral administration of ciprofloxacin and ampicillin on the resistome with a focus on the phage metagenome. They observed that the antibiotic treatment induced the SOS response, leading to the elevated abundance of AR genes in released viral particles (Modi *et al.*, 2013). The virome loaded with expanded AR genes potentially could transduce other resident or transient bacteria upon subsequent bacteriophage infection. Kim *et al.* (2016) examined the effects of bovine antibiotic growth promoters (bAGPs) on the propagation and spread of Shiga toxin (Stx)-encoding phages in *E. coli*. Co-culture of *E. coli* O157:H7 and other *E. coli* isolated from cattle in the presence of sub-lethal concentrations of bAGPs significantly increase the emergence of non-O157 but Stx-producing *E. coli* by triggering the SOS response system in *E. coli* O157:H7. Of a panel of bAGPs tested, ciprofloxacin, chlortetracycline, and oxytetracycline induced the most significant propagation of Stx phages (Kim *et al.*, 2016).

Stress hormone

Stress-related neurotransmitter hormones in the gut, such as norepinephrine (NE), can influence both the growth and virulence-associated features of a number of bacterial species (Lyte, 2011; Barrett *et al.*, 2012; Cryan and Dinan, 2012). Interestingly, a recent report also showed that NE could increase conjugative transfer of AR genes between a clinical strain of *Salmonella typhimurium* and an *E. coli* recipient strain *in vitro*; the greatest effect was observed at the physiologically relevant concentration of 5 mM of NE during acute host stress (Peterson *et al.*, 2011). Phentolamine, an α-adrenergic receptor antagonist, negated the effect of NE on conjugation more strongly than propranolol, a β-adrenergic receptor antagonist. This NE-mediated enhancement in conjugation is likely due to the significantly upregulated expression of plasmid-encoded transfer (*tra*) genes, which is necessary for conjugation, in the presence of NE (Peterson *et al.*, 2011).

Microbiota and derived factors

Recently, enhanced exchange of genetic material has been observed among the bacteria engulfed by amoebae (Moliner *et al.*, 2010). Protozoans can serve as a survival niche and protective shelter for high levels of foodborne pathogens (Tezcan-Merdol *et al.*, 2004; Olofsson *et al.*, 2013; Lambrecht *et al.*, 2015). Given its abundance in the gastrointestinal tract, particularly in the rumen, protozoans may provide a unique niche for efficient dissemination of AR genes between bacterial cells. Notably, the rumen ciliates have been shown to boost HGT between *Klebsiella* and *Salmonella*, both *in vitro* and *in vivo* (in the rumen), most likely via conjugation (McCuddin *et al.*, 2006). It was speculated that the close proximity of the donor and recipient, together with other stress conditions inside the ciliate, may contribute to the enhanced HGT between the different bacterial species (McCuddin *et al.*, 2006).

Recent studies also indicated that some microbiota-derived factors that involve quorum sensing could promote HGT. Quorum sensing is a bacterial density-dependent phenomenon mediated through production and release signal molecules (autoinducers) from bacteria to the extracellular environment (Fuqua *et al.*, 1994; Waters and Bassler, 2005). Once the concentration of autoinducer reaches the minimal threshold, bacteria respond with significantly altered gene expression profiles, leading to significant changes in behavior, physiology, and even virulence (Waters and Bassler, 2005). It has been reported that quorum sensing induced synchronous development of competence in *Pneumococcus* and *S pneumoniae* (Tomasz, 1965; Havarstein *et al.*, 1995). Bacterial pheromones, secreted peptides associated with quorum-sensing signaling pathway, were also observed to regulate conjugative plasmid transfer through intercellular signaling system (Dunny, 2013). Pheromone-responsive plasmids also have been shown to promote genome plasticity in antibiotic-resistant *Enterococcus faecalis* (Clewell, 2007; Dunny, 2007) as well as *Enterococcus faecium* (Heaton and Handwerger, 1995). Given that pheromone was prevalent in intestinal enterococci, quorum sensing may contribute significantly to HGT between *Enterococcus* spp. in the gut.

Conclusions

The gastrointestinal tract is a unique and ideal environment for HGT of AR genes, a programmed process playing a critical role in the development, transmission, and evolution of AR genes among bacterial organisms (same or different species). However, the process of HGT in the intestine is still largely unknown, particularly in terms of the specific factors promoting *in situ* HGT, which greatly impedes the development of effective AR mitigation strategies. This review summarized the findings from extensive *in vitro* studies and discussed the factors that potentially influence HGT processes in the intestinal tract, such as SOS response, stress hormone, microbiota and microbiota-derived factors. In the future, well-controlled animal studies are highly warranted to examine *in vivo* HGT processes and to evaluate the role of factors contributing to HGT in the

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intestine; such studies will generate new and important information for risk assessment and risk management of AR resistance, consequently developing practical and effective strategies to mitigate AR in animal production systems. For example, if a specific SOS-inducing antibiotic is demonstrated to greatly enhance HGT efficiency in the intestine, particularly via multiple HGT pathways (conjugation, transduction, or transformation) with respect to diverse AR genes, this antibiotic may be recommended for restricted use in food animals to reduce the risks of dissemination of AR genes and the emergence of AR pathogens.

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