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Mobile Genetic Elements in Streptococci

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Abstract

Streptococci are a group of Gram-positive bacteria belonging to the family Streptococcaceae, which are responsible of multiple diseases. Some of these species can cause invasive infection that may result in life-threatening illness. Moreover, antibiotic-resistant bacteria are considerably increasing, thus imposing a global consideration. One of the main causes of this resistance is the horizontal gene transfer (HGT), associated to gene transfer agents including transposons, integrons, plasmids and bacteriophages. These agents, which are called mobile genetic elements (MGEs), encode proteins able to mediate DNA movements. This review briefly describes MGEs in streptococci, focusing on their structure and properties related to HGT and antibiotic resistance.

Introduction

Streptococci are a group of Gram-positive bacteria widely distributed across human and animals. Unlike the *Staphylococcus* species, streptococci are catalase negative and are subclassified into the three subspecies alpha, beta and gamma according to the partial, complete or absent hemolysis induced, respectively. The beta hemolytic streptococci species are further classified by the cell wall carbohydrate composition (Lancefield, 1933) and according to human diseases in Lancefield groups A, B, C and G. group C and group G antigen are relatively rare compared with group A and group B antigen (Korona-Glowniak et al., 2015). Streptococci cause numerous diseases in human and animals, and many of them can cause serious and invasive infections damaging public health.

Group A streptococcus (GAS), also known as *Streptococcus pyogenes,* causes serious infections such as streptococcal toxic shock syndrome necrotizing fasciitis, and causes mild human infections such as impetigo and pharyngitis. Repeated GAS infections can cause autoimmune diseases, such as acute rheumatic fever, rheumatic heart disease and acute post-streptococcal glomerulonephritis (Parks et al., 2015). Group B streptococcus (GBS), also known as *Streptococcus agalactiae*, is included in the normal flora of the rectum and female urogenital tract, which under normal circumstances causes no harm, but it may trigger infection when the body defense system is weak. The infection can be transferred to a neonate from the mother colonized with GBS, causing meningitis and sepsis in newborns (Verani et al., 2010). The most represented specie belonging to the groups C and G streptococci is the *Streptococcus equisimilis,* a subspecies of *Streptococcus dysgalactiae*, which is usually present in human upper airways

(Jensen and Kilian, 2012). *S. dysgalactiae* is able to colonize other sites like skin, causing superficial and invasive infections such as septic arthritis, skin and tissue infections (Brandt and Spellerberg, 2009). *Streptococcus pneumoniae* is part of the normal flora of the human upper respiratory tract. The primary site of pneumococcal colonization in human body is the nasopharynx. It can cause a wide spectrum of infections, including invasive and non-invasive diseases. Pneumococcal infection can occur at multiple sites including the meninges and lung parenchyma, and is the primary cause of community acquired pneumonia (CAP) and a main cause of bacteremia, especially in infants and older adults (Remington and Sligl, 2014). The viridans streptococci, belonging to a heterogeneous group, are commensal flora of the oral cavity, upper respiratory, gastrointestinal and female genital tracts. These species can be divided into five groups named *Anginosus*, *Mutans*, *Mitis*, *Salivarius* and *Sanguinis* (Facklam, 2002). The viridians streptococci can cause severe infections including infective endocarditis, dental caries and deep seated abscesses (Menon, 2016). The *Streptococcus anginosus* group, previously known as *Streptococcus milleri*, includes three distinct species: *Streptococcus intermedius*, *Streptococcus anginosus* and *Streptococcus constellatus* (Claridge et al., 2001). These species, constituting the normal flora of the gastrointestinal tract and oropharynx, can form abscesses, which may alter the spectrum of related clinical disease. The most common infections caused by the *S. anginosus* group are empyema, infective endocarditis, cellulitis, and abscesses in the head, neck, lung and abdomen (Kowlessar et al., 2006; Noguchi et al., 2015).

Many diseases are caused by or associated with streptococci, but the treatment

options are limited. Currently, one of the biggest problems is the bacterial antibiotic resistance. It has been reported that some GAS isolates are resistant to macrolides, tetracycline and fluoroquinolones (Walker et al., 2014). Among GBS, several isolates resulted resistant to clindamycin and macrolides, and the percentage may be as high as 30% in some areas (Compain et al., 2014). The predominant antibiotic resistance of *S. dysgalactiae* subsp. *equisimilis* isolates are erythromycin, clindamycin and tetracycline (Lu et al., 2016).

Some bacterial strains have intrinsic resistance, while others gain antibiotic resistance by mutation, horizontal gene acquisition and recombination with foreign DNA. In most instances, these mechanisms occur together. Mutations can be considered the workhorse of bacteria evolution, while the horizontal gene transfer (HGT) can immediately transfer a resistant gene (Perry et al., 2014). HGT is the sharing of genetic material among organisms other than by the DNA transmission from parent to offspring, which enable organisms to acquire new genes and phenotypes.

Microbe employs different mechanisms to transfer genes horizontally, including transformation, transduction and conjugation. In natural transformation, cells uptake naked DNA from the environment. It was reported that there exist about 80 species capable of transformation, and they are evenly divided between Gram-positive and Gram-negative bacteria (Johnston et al., 2014). Transduction is a process in which foreign DNA is transferred into a cell with the help of a virus or viral vector, transferring virulence genes between bacteria. Physical contact

between the cell providing the DNA and the cell receiving the DNA is not required. Conjugation is the transfer of DNA between cells by a bridge-like connection or by direct cell-to-cell contact. During conjugation the cell donates a conjugative or mobilizable genetic element that is often a transposon or a plasmid. The result is frequently beneficial to the recipient, gaining xenobiotic tolerance, antibiotic resistance or new metabolic capability. Agents responsible of DNA movement in HGT are called mobile genetic elements (MGEs). Here, we reviewed principal MGEs in streptococci species, including transposable elements, integrons, plasmids and bacteriophages.

Transposable elements

A transposable element, is a segment of DNA that can be integrated in different sites along a chromosome, sometimes creating or reversing mutations and altering the cell genetic identity and genome size. Transposable elements were discovered by the American geneticist McClintock in 1951 (Korona-Glowniak et al., 2015), and were widely recognized in 1967 when Shapiro found transposable elements in *Escherichia coli*. Initially transposable elements were considered unusual (Fedoroff, 2012a), but then they were regarded as 'selfish DNA' being able to replicate autonomously. More evidences showed that transposons play a crucial role in genome evolution (Fedoroff, 2012b). Transposable elements are commonly found in chromosomes, integrative conjugal elements (ICEs), bacteriophages, and plasmids. In prokaryotes, transposons can mainly fall into two categories, the insertion sequence (IS) and transposon (Tn).

Insertion sequences

An insertion sequence, also known as insertion sequence element, or IS, is a short DNA sequence that acts as a simple transposable element. It is defined as a small (2.5-kb), phenotypically cryptic segments of DNA with a simple genetic organization, and capable of inserting at multiple sites in a target molecule (Mahillon and Chandler, 1998). ISs present two major characteristics: they are small compared with other transposable elements and they only encode proteins relevant for transposition activity. These proteins usually catalyze enzymatic reactions, help the ISs movements, and can also stimulate or inhibit the transposition activity. However, in many cases, the exact role and activity of the protein are still unclear (Siguier et al., 2014).

Many studies have been conducted about the relationship between ISs and the antibiotic resistance in *Streptococcus*. Beigverdi et al. isolated forty-one *S. agalactiae* from pregnant women at 35-37 weeks of gestation, and found that 97.6%, 24.4%, and 14.6% of them were resistant to tetracycline, erythromycin and clindamycin, respectively. The insertion sequence IS1548 was found in 63.4% of isolates, which flanking the antibiotic gene (Beigverdi et al., 2014). Another study showed that the presence of rib, IS861 and GBSi1 group II intron within the C5a peptidase gene might accentuate the group B *Streptococcus* virulence potential and ecological success (Florindo et al., 2014). Tn*Ss1* flanked by direct repeats of an IS6-family element may be related to the chloramphenicol resistance of *Streptococcus suis* (Takamatsu et al., 2003). In foodborne strains of *S. bovis*, there was a subregion of about 4 kb containing a novel mosaic tetracycline resistance gene *tet*(S/M) flanked by two copies of the IS1216 mobile

element (Barile et al., 2012).

Transposons

Transposons can be described as either composite transposons or complex transposons (Bennett, 2004). Composite transposons, for example Tn*5*, Tn*9* and Tn*10*, are flanked by two separate identical or different IS elements. A composite transposon contains protein coding sequences encoding resistance to one or more antibiotics. A complex transposon has a more complicated structure than a composite transposon, being usually flanked by short IRs (Mahillon and Chandler, 1998). Between IRs there are genes related to transposition and antibiotic resistance (table.1).

Table 1. Details of Transposons discussed in this review

Name	Approximate size (kb)	Antibiotic resistance gene
Tn*916*	16.4	*tet*(M)
Tn*917*	5.3	*tet*(M)and *erm*(B)
Tn*3872*	24	*tet*(M)and *erm*(B)
Tn*5801*	25.8	*tet*(M)
Tn*6002*	20.9	*erm*(B) and *tet*(M)
Tn*6003*	25.1	*tet*(M) and *erm*(B)
Tn*1545*	25.3	*erm*(AM), *aph*A-3 and *tet*(M)
Tn*2010*	26.4	*mega*, *tet* (M) and *erm* (B)
Tn*2009*	23.5	*mega*, *tet*(M)
Tn*1207.1*	7.2	*mef*(A)
Tn*1207.3*	52.5	*mef*(A)
Tn*2017*	28.5	*mega*, *tet*(M), *erm*(B)
Tn*5251*	18	*tet*(M)
Tn*5253*	64.5	*cat* and *tet*(M)
Tn*5252*	47.5	*cat* and *tet*(M)

Tn*916*

Tn*916* is a 16.4 kb transposon containing a tetracycline resistance determinant. Tn*916* can transfer through conjugation at frequencies of 10^{-8} to 10^{-5} per donor in the absence of plasmid DNA, and it is also capable of insertion at different locations of the recipient chromosome (Johnson and Grossman, 2015). Some of the trans-conjugants possess more than two non-tandem copies (Gawron-Burke and Clewell, 1982), and have been named “conjugative transposon”, or “integrative conjugative element”. The recombination module of all Tn*916*-related elements is located at one end of the element with imperfect repeat sequences. Tn*916* contains four functional modules: regulation, conjugation, recombination and accessory genes not involved in conjugative transposition (e.g. tetracycline resistance). The proposed regulatory region of Tn*916* includes *orf12*, *orf9*, *orf7* and *orf8*. *Orf6* and *orf10* are also in this region, but their functions are not clear yet (Figure.1) (Celli and Trieu-Cuot, 1998). Homologous segments have been found in the transposon junction regions but there was no direct duplication of the target sequence (Clewell et al., 1988). Tn*916* replicates autonomously *via* a rolling-circle mechanism, which depends on the relaxase encoded by *orf20* (Wright and Grossman, 2016). It has been reported that Tn*916* transposes by an excision-insertion mechanism (Caparon and Scott, 1989), in which it is firstly excised by a cut from donor cells and transformed into a circular form, then it is transferred by cell-cell contact, and finally it is inserted into receiving cells through recombinase (Roberts and Mullany, 2011). Two transposon-encoded proteins, Xis-Tn (an accessory protein) and Int-Tn (a tyrosine recombinase), are needed for excision. Int-Tn alone is enough for integration. The conjugative transposition of Tn*916* needs a functional integrase in both recipient and donor strain (Storrs et

al., 1991). The DNA sequences of the target sites of many Tn*916* insertions showed that Tn*916* prefers to use A-T rich targets in different hosts (Wang et al., 2000). The most important regulatory event in Tn*916* for conjugative transposition is the interaction between ribosomes and tetracycline, which makes the transcriptional terminator destroyed at the end of *orf12* and continues transcribing the *tet*(M) and the downstream genes. Tetracycline can inactivate most of the ribosomes, and a few ribosomes can remain active by the protection of the basal expression of *tet*(M) (Roberts and Mullany, 2009).

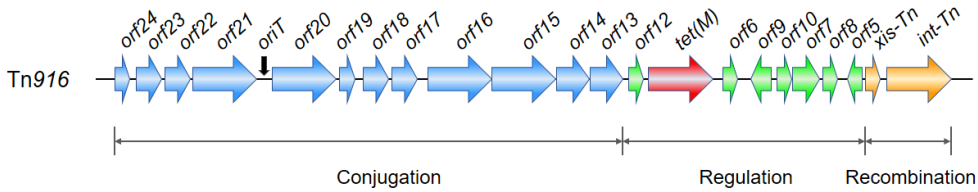

Figure 1. Schematic representation of Tn*916*.

The Tn*916* family is widespread among numerous commensal and pathogenic bacteria. Tn*916* has an extensive host range, both *in vitro* and *in vitro* (Scott, 2002). In *S. suis* serotype 2, the presence of Tn*916* with *tet*(M) has favored the pathogenicity and dissemination of this pathogen (Zhang et al., 2015). In 138 antibiotic resistant pneumococcal isolated strains from nasopharynx of healthy young children from Lublin of Poland, Tn*916* was detected in 16 strains (Korona-Glowniak et al., 2015). Moreover, among 62 tetracycline-resistant *tet*(M)-positive *S. agalactiae* isolate strains, 70% of them harboring Tn*916* (Mingoia et al., 2013).

Tn*917*

Tn*917* was identified on pAD2, a nonconjugative plasmid of *Enterococcus faecalis* strain DS16 (Tomich et al., 1980). Erythromycin resistance associated with Tn*917* is inducible, and the exposition of cells to low concentrations of erythromycin can induce transposition from pAD2 to pAD1. Tn*917* is 5,257 bp in length and has six open reading frames. *Orf2* is *erm*(B), which is responsible for erythromycin resistance. In 263 viridans group streptococci strains, the long-established transposon Tn*917* has been found in seven isolates by PCR mapping, which including one *Streptococcus mitis* strain, three *Streptococcus oralis* strains, two *S. sanguinis* strains and one *S. anginosus* strain (Brenciani et al., 2014).

Tn*3872*

Tn*3872* is found by DNA-DNA hybridization, and nucleotide sequence analyses revealed that they were made up by the insertion of Tn*917* into *orf9* of Tn*916* (McDougal et al., 1998). With the *tet*(M) gene of Tn*916* linked to the *erm*(B) gene of Tn*917*, Tn*3872* can lead to the erythromycin and tetracycline resistance. *Tet*(M) on the Tn*3872*-like transposon in the *S. agalactiae* isolates has been confirmed by PCR with the primers *tet*(M)-forward and *erm*(B) (Sadowy et al., 2010). Thirteen strains had Tn*3872* among twenty-one clinical *Streptococcus salivarius*, and two of them carried a Tn*3872* with a silent *tet*(M) gene (Chaffanel et al., 2015). In pneumococcus, *tet*(M) can evolve through homologous recombination after Tn*3872* integration into the host chromosome (Chaffanel et al., 2015). It has been reported that Tn*916* can transfer from an enterococcal donor to a pneumococcal recipient, but the composite transposon Tn*3872* cannot transfer from a pneumococcal to another pneumococcal or from an enterococcal to a pneumococcal. The low transfer frequency of Tn*3872* or the inactivation of

mobility of Tn*916* due to the insertion of Tn*917* may be the reason (Swartley et al., 1993). However, in *Streptococcus defectives* strains, the frequency of conjugative transposition of Tn*3872* was ranging from $4x10^{-6}$ to $2.8x10^{-8}$ (Poyart et al., 2000). Tn*3872*-related elements were distributed in many serotypes of pneumococcus, i.e. 6B, 23F, and 14 (Quintero et al., 2011; Ramos et al., 2014). In some strains, Tn*3872* and Tn*3872*-related elements were lacking *tet*(M) gene (Cochetti et al., 2007; Quintero et al., 2011). Genetic elements relevant to drug resistance can insert in Tn*3872*, and the strain will display resistance to other antibiotics. For example, *mega*, a genetic element carrying *mef*(E), has been found transferred by conjugation to *S. pneumoniae* and then linked with Tn*3872*-like transposon by recombinases (Figure.2) (Del Grosso et al., 2006; Santagati et al., 2009). In some strains, Tn*3872* carries transposase genes *tnpA* and *tnpR* (Korona-Glowniak et al., 2016; Korona-Glowniak et al., 2015).

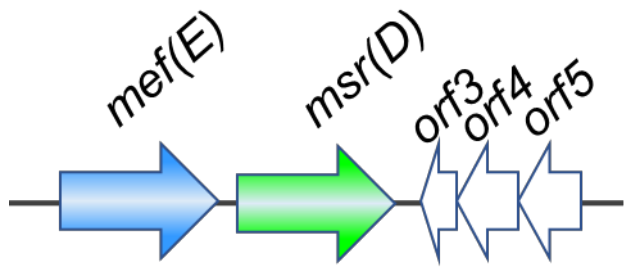

Figure 2. *Mega* of *S. pneumoniae.*

Tn*5801*

Tn*5801*, 25.8 kb in size, was originally detected in *Staphylococcus aureus* Mu50 (Kuroda et al., 2001). It is a Tn*916* family element, and the *int* and *xis* genes of Tn*916* are replaced by a unique gene (*int5801*) encoding different types of tyrosine recombinases. The *tet*(M) genes of Tn*5801* and Tn*916* are similar, with

about 97.7% DNA identity (de Vries et al., 2016). Tn*5801*-like transposons have been detected in some streptococci, including *S. mitis* B6 (Denapaite et al., 2010), S. *agalactiae* (Da Cunha et al., 2014; Mingoia et al., 2013) and *S. oralis* (Brenciani et al., 2014). In *tet*(M)-positive tetracycline resistant *S. agalactiae*, 30% harbors Tn*5801*-like element (Mingoia et al., 2013).

Tn*6002*

Tn*6002*, a 20.9 kb element, is formed by the insertion of an *erm*(B)-carrying DNA fragment into Tn*916*, which is located between *orf20* and *orf19* (Brenciani et al., 2007). It was originally discovered in *Streptococcus cristatus* and designated as Tn916Erm, whose complete sequence is available in GenBank since 2005 (accession no. AY898750) (Varaldo et al., 2009). It has also been found in *S. pyogenes* (Ardanuy et al., 2010; Brenciani et al., 2007), *S. salivarius* (Chaffanel et al., 2015) and S. *pneumoniae* (Calatayud et al., 2007; Korona-Glowniak et al., 2016; Quintero et al., 2011). In *erm*(B)-positive pneumococci, Tn*6002* was the most common *erm*(B)-carrying Tn*916*-related element (Cochetti et al., 2008; Korona-Glowniak et al., 2015). The spread of Tn*6002* from the ST386/ST4310/ST4825 lineage to the serotype 6C of the pneumococci isolates lead to the increase of the antimicrobial non-susceptibility of serotype 6C in the late-PCV7 period (Rolo et al., 2011). In viridans group streptococci (VGS), Tn*6002* were also detected into *S. mitis* and *S. oralis* (Brenciani et al., 2014). In oral streptococci isolated from healthy human, Tn*6002* was detected from *Streptococcus infantis*, *S. mitis*, *S. sanguinis*, *Streptococcus australis*, *S. oralis* and *Streptococcus parasanguini*.

Tn*6003*

Tn*6003*, a new Tn*6002*-related composite element, found in 2007 (Cochetti et al., 2007), contains *tet*(M) and *erm*(B) gene, as well as the *aadE-sat4-aphA*-3 gene cluster that forms a 4.2 kb fragment called MAS (macrolideaminoglycoside-streptothricin). Tn*6003* is 25.1 kb (accession number: AM410044). The transfer frequency of Tn*6003* from *S. pneumoniae* strain Ar4 to *E. faecalis* JH2-2 is $1.7x10^{-7}$ (Cochetti et al., 2007). Tn*6003* is a common Tn*916*-related element in *erm*(B)-positive pneumococci (Quintero et al., 2011).

Tn*1545*

Tn*1545*, a 25.3 kb element, was originally detected in the chromosome of *S. pneumoniae* BM4200 (an antibiotics-resistant clinical isolates) (Courvalin and Carlier, 1987). It confers resistance to erythromycin (*erm*AM), kanamycin (*aphA*-3) and tetracycline (*tet*M) and was the first genetic element found carrying the *erm*(B) gene in *S. pneumoniae*. Compared with Tn*916*, between o*rf20* and *orf19*, Tn*1545* showed the insertion of two *erm*(B) and MAS elements; and between *orf13* and *tet*(M), Tn*1545* had the insertion of IS*1239* (Quintero et al., 2011). Tn*1545* is self-transferable to various streptococci, including *Streptococcus cremoris*, *S. sanguinis*, *Streptococcus lactis*, *Streptococcus diacetylactis*, *and E. faecalis*. It is a conjugative shuttle transposon, which can transpose from the hybrid plasmid to various new sites of the new host chromosome and then re-transpose from the chromosome to the plasmid at a frequency of $5x10^{-9}$. Though the absence of recombinase genes, the MAS element of Tn*1545* is able to exploit

long flanking repeats for excision in a circular form (Palmieri et al., 2012).

Tn*2010*

Tn*2010*, 26.4 kb in length, is a composite element carrying both *mega*, *tet*(M) and *erm*(B) genes (Del Grosso et al., 2007; Talebi et al., 2016). Tn*2010* is similar to Tn*6002*, including sequence and structure, and it has the insertion of the *mega* element into *orf6* of the Tn*6002,* as well as 70 nucleotide mutations (Li et al., 2011). It has been indicated that Tn*2010* could not transfer macrolide resistance through conjugation (Del Grosso et al., 2006). Zhou et al. proved that Tn*2010* is more likely to be transferred by transformation other than conjugation in *S. pneumoniae*, and the transformation frequency is $3x10^{-7}$ transformants per cfu (Zhou et al., 2014).

Tn*2010* is common in *S. pneumoniae* (Bowers et al., 2012; Korona-Glowniak et al., 2015), and recently it has been found in *S. mitis* (Brenciani et al., 2014). In *S. pneumoniae*, most strains owning Tn*2010* belonging to serotype 19A or 19F (Korona-Glowniak et al., 2016). The wide spreading of the macrolide resistance genes is highly relevant to Tn*2010*. *S. pneumoniae* A026, isolated from cerebrospinal fluid, contains Tn*2010* and is resistant to penicillin, trimethoprim, erythromycin, sulfonamides, and tetracycline but is susceptible to levofloxacin (Sui et al., 2013).

Tn*2009*

Tn*2009* is a composite element of approximately 23.5 kb, originally found in the chromosomes of the two *S. pneumonia* strains (Del Grosso et al., 2004). Subsequently, it was detected in *S. mitis* (Brenciani et al., 2014), *S. pyogenes* (Del Grosso et al., 2011) and *S. salivarius* (Chaffanel et al., 2015). Tn*2009* has the insertion of the *mega* element into the *orf6* of a Tn*916*-like transposon containing *tet*(M). In many Tn*2009* element of *S. pyogenes* strains, *mef*(E) is included in a typical *mega* element, while in some strains, *mef(E)* is physically linked with *tet*(M) (Del Grosso et al., 2011). Tn*2009* contains determinants for tetracycline, macrolide and erythromycin resistance. In *S. pneumoniae* and *S. pyogenes* the conjugal transfer of Tn*2009* has not been detected, unlike the transfer by transformation (Del Grosso et al., 2016; Del Grosso et al., 2004). It has been reported that the conjugative Tn*2009* in a Gram-negative commensal bacteria *Acinetobacter junii 326* was indistinguishable from the *S. pneumoniae* Tn*2009* element (Ojo et al., 2006).

Tn*1207.1*

Tn*1207.1*, 7,244 bp in size, is the first described *mef*-carrying genetic element. It has been found in both *S. pneumoniae* and *S. pyogenes* (Santagati et al., 2000). Tn*1207.1* consists in first 5 ORFs with the same direction of transcription and the last 3 ORFs in the opposite direction (Pozzi et al., 2004). The *orf2* encodes a site-specific recombinase, while *orf5*, starting from the downstream of the *mef* (A), is homologous to *vga*(A) and *msr*(SA) (Pozzi et al., 2004). The *mrs*(SA) and *mef*(A) are two important parts of the *mega* element. Tn*1207.1* is not a conjugative element (Santagati et al., 2000).

Tn*1207.3*

Tn*1207.3*, homologous to *Tn1207.1*, is resistant to macrolides and carries the *mef*(A) gene. Tn*1207.3* was found in *S. pyogenes*, *S. pneumoniae*, S. *dysgalactiae* subsp. *Equisimilis* (Figueiredo et al., 2006; Marimon et al., 2005; Rato et al., 2010), *S. gordonii* (Santagati et al., 2003) and *S. agalactiae* (Figueiredo et al., 2006; Marimon et al., 2005). Its nucleotide sequence is highly identical to that of Tn*1207.1,* and only the right end of the Tn*1207.3* has a new sequence (Pozzi et al., 2004; Santagati et al., 2003). Tn*1207.3* can transfer from *S. pyogenes* to *S. pneumoniae* by conjugation, and from *S. pneumoniae* trans-conjugant MF2 to *S. pyogenes* and *S. gordonii* (Santagati et al., 2003). The transfer frequency of Tn*1207.3* to various *S. pyogenes* is significantly different, and the mean frequencies range from $7.20x10^{-8}$ to $1.13x10^{-6}$ conjugant per recipient (Hadjirin et al., 2014). The integration of Tn*1207.3* did not cause deletions, while integration of Tn*1207.1* caused a 1947 bp deletion (Pozzi et al., 2004). The DNA sequencing of Tn*1207.3* indicated that it is actually a prophage, the same with the right end of ϕ10394.4 (Iannelli et al., 2014a).

Tn*2017*

Tn*2017*, a Tn*916*-like element, was found in *S. pneumoniae* and beta-hemolytic *Streptococcus* (Korona-Glowniak et al., 2015). Tn*2017* resembles Tn*3872*, which is the insertion of *mega* element into the *orf6* of Tn*3872*, thus being resistant to erythromycin, tetracycline and macrolide. Tn*2017* also contains the *tnpA* and *tnpR* genes. In erythromycin and tetracycline resistant beta-hemolytic

Streptococcus isolates, Tn*2017* is the most prevalent transposon, while in *S. pneumonia* isolates it is the second most common transposon (Tantivitayakul et al., 2016).

Tn*5251*

Tn*5251*, 18 kb in length, is like Tn*916* in structure and size. It contains 22 ORFs, of which 20 have the same direction and transcription, and half are annotated (Santoro et al., 2010). Tn*5251* carries *tet*(M), *int* and *six* genes. Tn*5251* cannot transfer independently from Tn*5253* through conjugation (Kilic et al., 1994), however recently it has been proposed that Tn*5251* can transfer autonomously in *S. pneumoniae* and *E. faecalis*, and the autonomous copies can moved into *S. pneumoniae*, *S. gordonii*, *S. agalactiae*, *S. pyogenes*, *E. faecalis* and *Bacillus subtilis* (Santoro et al., 2010).

Tn*5253*

Tn*5253*, originally found in the chromosome of *S. pneumoniae* BM6001 (Dang-Van et al., 1978), was formerly called the Ω(*cat-tet*) element, carrying the *cat* and *tet*(M) genes (Vijayakumar et al., 1986). Tn*5253* is 64.5 kb in size, and contains 79 ORFs in total, 38 of which have annotations (Iannelli et al., 2014b). Tn*5251* and Tn*5252* were found integrated into the Tn*5253*, demonstrating that Tn*5253* was a composite element (Ayoubi et al., 1991). The highest conjugation frequency of Tn*5253* was 6.7×10^{-3} trans-conjugants/donor when S. *pyogenes* was the donor (Iannelli et al., 2014b). Tn*5253* is common in the drug resistance of *S. pneumoniae* strains, especially in the serotype 6B strains, but the *cat* gene may

not be detected for the modifications of the transposon (Quintero et al., 2011). Among clinical isolates of *S. pneumoniae*, Tn*5253*-related elements are extremely different (Henderson-Begg et al., 2009; Mingoia et al., 2011). In streptococci, *cat*(Q) and *mef*(I) are linked in a genetic module called IQ module that has been detected in *S. pneumoniae*, *S. pyogenes* and viridans group streptococci. The IQ module in *S. pyogenes* belongs to the Tn*5253* family (Mingoia et al., 2014a; Mingoia et al., 2014b; Mingoia et al., 2007).

Tn*5252*

Tn*5252*, 47.5 kb in size, is a streptococcal conjugative transposon (Kilic et al., 1994). It was originally found in the chromosome of *S. pneumoniae* BM6001 as a part of Tn*5253* (Shoemaker et al., 1979). Until now, Tn*5252* has only been partially sequenced, showing the presence of an integrase gene, *tet*(M), and *cat*. While Tn*916* class of element can insert at several spots, Tn*5252* prefer to integrate at a specific site, which is drove by *int* gene. *Cat* gene can encode chloramphenicol acetyltransferases (CATs) that lead to the enzymatic inactivation of the drug, and Tn*5252* can be detected in the clinical chloramphenicol resistant strains (Korona-Glowniak et al., 2015). Since the discovery of Tn*5252*, its homologues have been detected in many streptococcal species, such as *S. suis*, *S. pneumoniae* and GBS (Kayansamruaj et al., 2015; Wyres et al., 2013).

Integron

Integrons are genetic elements that can help bacteria to enhance their ability of adaptation and evolution through the acquisition, stockpiling, excision and

differential expression of new genes (Escudero et al., 2015). The genes are usually contained in a gene structure called cassette, a circular element carrying one promoterless gene together with a recombination site (*attC*) (Figure.3). Another structure included in integrons is a stable platform. The platform has several parts, and the first is *intI*, a gene encoding the integrase, which is a member of tyrosine recombinase family (Messier and Roy, 2001). The second part is the *attI* site, an integron-associated recombination site, which is in the upstream of the *intI* gene (Partridge et al., 2000). The third part is *Pc*, an integron-associated promoter, located between the *intI* and the *attI* site or within the *intI* gene (Collis and Hall, 1995). Cassettes are non-replicative elements, and they play their roles by integrating into the platform and expression from the *Pc* promoter. With the constant integration taking place, an array of cassettes will be assembled, and the cassettes' excised and integrated into the first position have the maximal expression (Escudero et al., 2015). There are two key advantages owning by the integron system for the genomic studies. First, the new cassette is integrated into the bacterial genome at *attI* site, which will not disturb the existing genes. Second, the newly integrated cassettes are expressed by the promoter *Pc* that it is ready for the natural selection (Gillings, 2014).

At present, there are five classes of mobile integrons associated with antibiotic resistance: classes 1, 2 and 3, obtained from clinical contexts (Partridge, 2011); class 4, detected in *Vibrio cholerae*; and class 5, found on the plasmid of *Alivibrio salmonicida* (Gillings, 2014). All five classes are linked to mobile elements, such as conjugative plasmids, insertion sequences and transposons, which can work as vehicles for the gene transmission (Mazel, 2006).

Integrons play an important role in the spread of antibiotic resistance, especially

in Gram-negative pathogens. Nonetheless, recent studies focused on the role of integrons in antibiotic resistance in Gram-positive microorganisms (Deng et al., 2015; Li et al., 2017), such as *Staphylococcus, Enterococcus, Corynebacterium* and *Streptococcus*. In streptococci, *S. pneumoniae* was proved to carry class 1 integron (Shi et al., 2006).

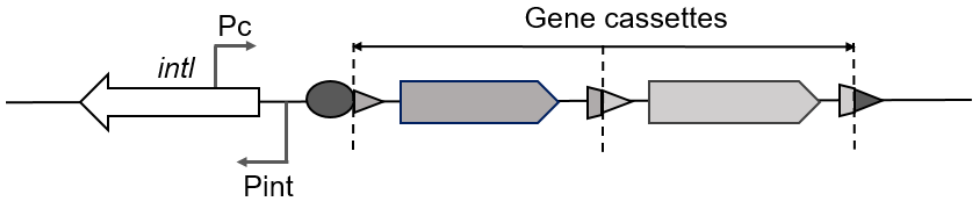

Figure 3. General structure of class 1 integrons.

Plasmids

Plasmids are a group of extrachromosomal DNA molecules that can self-replicate, are usually circular and does not contain genes required for essential cellular functions. The typical plasmid is a circular, closed double-stranded DNA molecule; nonetheless, linear double-stranded plasmids have been found (Stewart et al., 2005). In general, a plasmid includes essential genes related to replicative functions and accessory genes encoding different functions (Fernandez-Lopez et al., 2006) including drug resistance, fitness and virulence factors (Gyles and Boerlin, 2014). Plasmids are inherited from parent to daughter cells at cell division by partitioning. For some plasmids carrying accessory genes, they are therefore large and always present in low copy numbers in their host to ensure that the metabolic burden of maintaining and duplicating their genomes are not excessive. And the low copy number would increase their chance of being faithfully transmitted to daughter cells during division. Therefore, in order to decrease

random losses, the plasmid should be inherited stably at a short time (Sengupta and Austin, 2011).

Some plasmids carry genes allowing them to transfer by conjugation, while others are lacking such genes and count on the transferable function of other plasmids (Smillie et al., 2010). The conjugative genes trigger DNA transfer from the donor cell to the recipient cell, and other genes guarantee the DNA survival in the new host. The transfer genes also encode proteins able to 'pump' the DNA into recipient cells, and these proteins all belong to the TraG-like family of ATPases (Schroder et al., 2002). Relaxase are also important in many conjugative systems that can nick DNA to give a single-stranded substrate suitable for transfer, and the nicking process takes place in a specific site. In Gram-negative bacteria the pilus is an important section of conjugative systems. Pilus assembly plays the role of a type IV secretion system (T4SS) (Lybarger and Sandkvist, 2004), which help bacteria in two aspects**:** the genetic exchange and the transmission of effector molecules to target cells (Cascales and Christie, 2003).

In Gram-positive bacteria conjugative mechanisms include the pheromone-independent system that have a cell surface protein able to trigger mating-pair formation and pheromone-dependent mating systems in enterococci (Frost et al., 2005). In streptococci, the transfer of chromosomal DNA may be affected by the plasmid transfer in horizontal gene transfer, due to the chromosome mobilization ability of plasmids and the high frequency of recombination mode of the F plasmids (Lawley et al., 2003). Plasmids have been grouped according to their

replicons, and the streptococcal plasmid pMV158 belongs to the Rep_2 family (PF01719). There are several parts related to the mobilization of plasmid pMV158: prototype of the MOBV relaxase family, the product of the *mobM* gene, and its cognate origin of transfer, *oriT*. The *mobM* gene encodes the relaxase MobM, the relaxase of pMV158. MobM forms a dimer through its C-terminal domain, anchoring the protein to the cell membrane and participating in T4SS protein-protein interactions (Fernandez-Lopez et al., 2013). Plasmid pLS1 is a deleted derivative of pMV158, which has lost the ability of conjugative mobilization, thus decreasing the host cell growth by 8-9% in *S. pneumonia* (Hernandez-Arriaga et al., 2012). Many toxins and drug resistance genes in streptococci are encoded by plasmid. The β plasmid from *E. faecalis* can transfer the erythromycin and lincomycin resistance genes by conjugation (LeBlanc et al., 1978). The diplococci-producing ability can be transferred from the plasmid of *Streptococcus cremoris* 346 to the plasmid-free S*. cremoris* by conjugation with high frequency (Davey, 1984). The minocycline resistance in an oral S*treptococcus infantis* isolate may be due to the *tet(S)* gene which is carried by a novel low copy number plasmid (Ciric et al., 2014).

Bacteriophages

Certain drug resistance in bacteria are found to be relevant to phages (Brussow et al., 2004; Lekunberri et al., 2017), and there are many antibiotic resistant genes carried by the bacteriophages, such as *tetA*, *tetW*, *intI1*, *intI2*, *intI3*, *blaOXA-2* and *blaTEM* (Anand et al., 2016). Phage genomes may contain DNA or RNA, and the DNA can be single- or double-stranded. The essential genes of phages include the replicase genes, genes encoding for proteins involved in the DNA packaging,

and genes encoding phage components used to disturb the host cell replicative machinery (Frost et al., 2005).

Phage-mediated horizontal gene transfer occurs via lysogenic conversion or transduction. Moreover, some phages can play a significant role in the emergence of pathogens, including *S. aureus* (food poisoning), *S. pyogenes* (scarlet fever), *E. coli* (Shiga toxin) and *Clostridium botulinum* (botulism). In *S. mitis*, phage tail genes *pblA* and *pblB* encode adhesion proteins for bacterial host attachment. The process of phage DNA packaging is highly accurate, but the host genome DNA fragment may be packaged at a finite frequency compared with the phage DNA, resulting in phage particles that can attach and deliver the DNA into suitable bacteria, with the injected exogenous bacterial DNA integrated into the genome. The observation of toxin genes in *S. pyogenes* and *Corynebacterium* located next to the phage attachment site raised the hypothesis that the prophage genes actually stand for bacterial genes obtained by a false excision from a previous bacterial host (Brussow et al., 2004).

There are likely many new variants of MGEs, which can contain other resistant genes able to transfer to broad host range. Besides, MGEs may evolve complicated mechanisms of transcriptional regulation which likely minimize the biological cost to the host. Furthermore, many of the current studies are focus on laboratory strains, which may not reflect the variability of natural isolates. Therefore, it is urgent to clarify the structure and function of the DNA integrated in a new host, the rate of the gene transfer in both laboratory and natural bacteria,

as well as the effects of MGEs on the transcriptional regulation of the gene expression.

Conclusions

Here, we have reviewed principal MGEs in streptococci, including transposable elements, integrons, plasmids, and bacteriophages. MGEs have a strong environmental and clinical impact due to their role in the spread of antibiotic resistance in different bacteria. Transposable elements, mainly containing IS and transposons, are strictly related to the resistance to erythromycin, tetracycline, kanamycin and macrolide. The relationship between antibiotic resistance and integrons is well studied. Recently class 1 integron has been found in *S. pneumoniae*. Many toxins and the spread of drug resistance in *Streptococcus* are plasmid encoded, including the resistance to erythromycin, minocycline, and lincomycin. Finally, phages can mediate horizontal gene transfer *via* lysogenic conversion or transduction.

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