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# Phylogenetic signature of lateral exchange of genes for antibiotic production and resistance among bacteria highlights a pattern of global transmission of pathogens between humans and livestock

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ABSTRACT

The exchange of bacterial virulence factors driven by lateral gene transfer (LGT) can help indicate possible bacterial transmission among different hosts. Specifically, overlaying the phylogenetic signal of LGT among bacteria onto the distribution of respective isolation sources (hosts) can indicate patterns of transmission among these hosts. Here, we apply this approach towards a better understanding of patterns of bacterial transmission between humans and livestock. We utilize comparative genomics to trace patterns of LGT for an 11-gene operon responsible for the production of the antibiotic nisin and infer transmission of bacteria among respective host species. A total of 147 bacterial genomes obtained from NCBI were determined to contain the complete operon. Isolated from human, porcine and bovine hosts, these genomes represented six *Streptococcus* and one *Staphylococcus* species. Phylogenetic analyses of the operon sequences revealed a signature of frequent and recent lateral gene transfer that indicated extensive bacterial transmission between humans and pigs. For 11 isolates, we detected a Tn916-like transposon inserted into the operon. The transposon contained the *tetM* gene (tetracycline resistance) and additional phylogenetic analyses indicated transmission among human and animal hosts. The bacteria possessing the nisin operon and transposon were isolated from hosts distributed globally. These findings possibly reflect both the globalization of the food industry and an increasingly mobile and expanding human population. In addition to concerns regarding zoonosis, these findings also highlight the potential threat to livestock worldwide due to reverse zoonosis.

## 1. Introduction

Exposure to pathogens in areas where humans closely interact with livestock can cause zoonoses, which can impact human health and livestock productivity (Maudlin et al., 2009; Zhang et al., 2016). Livestock and humans can serve as reservoirs for bacteria and two-way transmission between animals and humans can affect pathogen evolution (Berngruber et al., 2013; Brown et al., 2012). For example, zoonotic transmission has been linked to the rise of several emerging infectious diseases (Grace et al., 2012). Transmission among hosts is mainly driven by consumption of contaminated food and water, biological vectors (i.e. pollen and insects) and by direct contact between humans and livestock (Clasen et al., 2015; Craft, 2015; Klous et al., 2016; Mann et al., 2015; Perkins et al., 2013; Reiner et al., 2013; Stull et al., 2013; van der Wielen and van der Kooij, 2013).

With the development of bacterial typing techniques, bacterial transmission between human and livestock populations has mainly been investigated by the following approaches: identical capsular typing (Manning et al., 2010), multi-locus sequence typing (Neyra et al., 2014), restriction fragment length polymorphism typing (Ocepek et al., 2005), random amplified polymorphic DNA typing (Singh et al., 2006), serotyping (Monno et al., 2009), pulsed-filed gel electrophoresis typing (Gilpin et al., 2008) and antibiotic susceptibility profiles (Pelkonen et al., 2013). These techniques are often combined with population genetic analyses (Rwego et al., 2008). However, a drawback to these approaches is the limited resolution of the marker employed. Whole genome sequencing offers the potential to utilize much larger nucleotide markers with far more resolution.

The acquisition of new genetic material can occur through lateral gene transfer (LGT), a crucial evolutionary mechanism for bacterial adaptation (Kingston et al., 2017; Price et al., 2012; Zhang and Lin, 2012). Evidence for LGT can be detected using phylogenetic methods (David and Alm, 2011; Lawrence and Retchless, 2009). Within a gene phylogeny, if the gene is following a pattern of vertical inheritance,

*Abbreviations:* API, average pairwise identity; ICE, integrative conjugation element

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strains of the same species should be monophyletic. However, when gene sequences for strains of the same species do not form monophyletic groups and some sequences group very closely or are even identical to sequences from distantly related species, these genes are deviating from a pattern of vertical inheritance and LGT is the likely explanation. Cells need to be proximate for the exchange of genetic material to occur; consequently, a phylogenetic signal of LGT between bacteria isolated from different hosts/environments can be used to infer transmission between these hosts/environments (Juhas et al., 2009; Sela et al., 2016; Yang et al., 2015). Allowing for a molecular clock, the accumulation of nucleotide differences (genetic distance) between sequences is correlated with time. Therefore, as the genetic distance between sequences increases, there is an increased possibility for intermediate hosts. However, bacteria mutation rates can be relatively high. For example, a recent study of *E. coli* estimated the rate of accumulation of nucleotide substitutions could be as high as 62 per thousand generations for an adapting population (Wielgoss et al., 2013). Consequently, one or two SNPs could accumulate over a very short period of time minimizing the possibility of intermediate hosts.

Virulence factors can offer competitive advantages allowing pathogens to outcompete other microbes within the same environment and influence transmission among hosts (Wickham et al., 2007). Furthermore, the ability of bacteria to either mutate or acquire new genes relating to virulence has the potential to help bacteria rapidly adapt to new environments and colonize new hosts (Sitkiewicz et al., 2006). Antimicrobials such as cytolysin (Tang and van der Donk, 2013), staphyococcin (Rogolsky and Wiley, 1977), pneumolysin (Li et al., 2017; Rossjohn et al., 1998), and hemolysin (Dinges et al., 2000) are virulence factors (Doran et al., 2002; Pritzlaff et al., 2001) that aid in bacterial colonization due to their lantibiotic capacity to form pores in cell membranes and inhibit cell wall biosynthesis of competing bacteria (Breukink et al., 1999; Wiedemann et al., 2001). Another lantibiotic, nisin, which has been shown to be closely related to these virulence factors, has the same antimicrobial capacity (Zhang et al., 2012). Indeed, bovine challenge experiments for *Streptococcus uberis* have shown nisin producers to outcompete non-producers (Pryor et al., 2009). Nisin is produced and regulated by an 11-gene operon (Fig. 1). In brief, production is initiated by the generation of a precursor molecule, which is encoded by *nisA*, the precursor is modified via dehydration (*nisB*), cyclization (*nisC*), and cleavage (*nisP*). The mature molecule (32 amino acids) is then transported out of the cell (*nisT*). Production is regulated by *nisRK*, and self-immunity to nisin is obtained via *nisI* and *nisFEG* (AlKhatib et al., 2014; Engelke et al., 1994; Field et al., 2015; Immonen and Saris, 1998; Kaletta and Entian, 1989; Ortega et al., 2015; Qiao and Saris, 1996; van der Meer et al., 1993; Ye et al., 1995).

Originally isolated from *Lactococcus lactis*, nisin has a broad range of effectiveness against Gram-positive bacteria including food-borne and spoilage pathogens such as *Staphylococci*, *Bacilli*, *Clostridia*, and *Listeria* (Jack et al., 1995; Kuwano et al., 2005). Consequently, it is often utilized as a food preservative (Field et al., 2015). Subsequent to *L. lactis*, additional species have been shown to possess the operon; for example, *Streptococcus suis, Streptococcus agalactiae,* and *S. uberis* (Richards et al., 2011; Thurlow et al., 2012; Wirawan et al., 2006; Wu et al., 2014). *S. suis* is an important zoonotic pathogen causing respiratory tract infections in porcine and humans (Goyette-Desjardins et al., 2014). *S. agalactiae* has a broad host range including human and bovine. In humans, it is responsible for severe neonatal infection. In bovine, it is a major causative agent of bovine mastitis (Delannoy et al., 2013; He et al., 2016). *S. uberis* is another leading cause of bovine mastitis, but is a strict animal pathogen (Cullen and Little, 1969; Di Domenico et al., 2015; Tassi et al., 2013; Zadoks et al., 2003).

In this study, we utilize the nisin operon as a high-resolution molecular maker to assess bacterial transmission among humans and livestock. We provide evidence for extensive pathogen transmission between human and animal hosts for numerous bacteria species worldwide. Furthermore, transmission patterns are not restricted by geographic distance, possibly reflecting the globalization of the food industry and mobility of the human population; with the later highlighting a somewhat overlooked threat to livestock via reverse zoonosis (human to animal transmission).

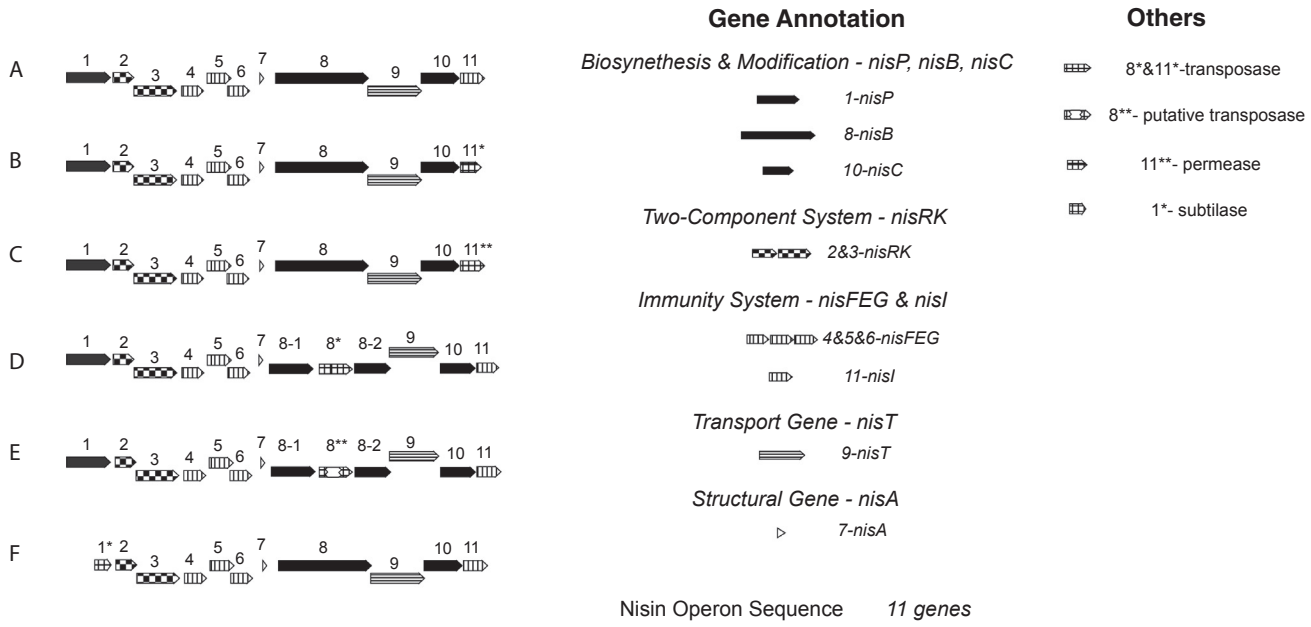


**Fig. 1.** Gene content and arrangement for the nisin operon. Each arrow represents a gene or genetic variant found within the operon, which are also labeled in the key. Sequence A shows the BLAST query sequence (human *Streptococcus agalactiae* GB00984). Sequence B represents one nisin operon from *Streptococcus suis* where the *nisI* gene at the 3′ end was replaced by a transposase. Sequence C illustrates six nisin operons for *Staphylococcus aureus* (human isolates) where the *nisI* gene was substituted by a permease. Sequence D represents one nisin operon from *Streptococcus agalactiae* (bovine isolate) where *nisB* was fragmented by an inserted transposase. Sequence E represents three nisin operons from *Streptococcus agalactiae* (bovine isolate) where each operon was distributed on two separate contigs. When the respective contigs were paired, there was a gap in *nisB*. Sequence F shows one nisin operon from a *Streptococcus hyovaginalis* porcine isolate where the *nisP* gene at the 5′ was replaced by a subtilase.

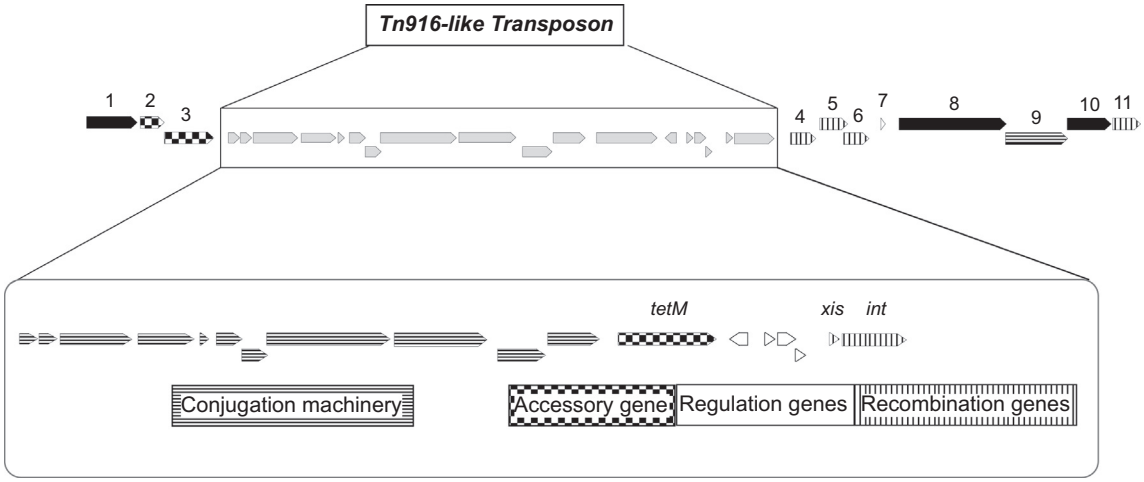


**Fig. 2.** Gene content and arrangement for the Tn916-like transposon inserted into the nisin operon for 11 *S. suis* (porcine isolates). Top of the figure shows the nisin operon with the transposon. Here, each black/patterned arrow represents a gene in the nisin operon, whereas grey arrows indicate genes in the Tn916-like transposon that were inserted between *nisK* and *nisF*. The transposon contained 18 genes (~18 kbps, represented at the bottom of the figure). Horizontal striped genes were annotated with conjugation machinery function, white genes were annotated with regulation function; *xis* and *int* were annotated as recombination genes, and the white checkered gene was annotated as *tetM*, an antibiotic resistance accessory gene.

## 2. Materials and methods

### 2.1. BLAST and phylogenetic analyses

The distribution of the nisin operon in bacterial genome sequences was assessed by performing a BLASTn search with an E-value cut-off of 1e−5. The query was the nisin operon from human *S. agalactiae* GB00984. The following databases were utilized for BLASTn searching: nonredundant nucleotide collection (nt/nr), reference sequence collection (refseq), whole genome shotgun contigs (wgs), and a database built using *S. agalactiae* short sequence reads downloaded from the NCBI sequence read archive (SRA). These reads were assembled into contigs using SPAdes v3.1.1 (Bankevich et al., 2012) and annotated using Prokka v1.11 (Seemann, 2014). In total, there were 439 hits, of which 426 were from NCBI databases and 13 from the unpublished *S. agalactiae* database. The nisin operon sequences were aligned using MAFFT v7.017 (Katoh et al., 2002) with default parameters as implemented in Geneious v8.1.8 (Kearse et al., 2012). Using FaBox (Villesen, 2007), the sequences were collapsed into haplotype sequences and used for phylogenetic reconstruction. This and two additional phylogenies (see Results and Discussion) were constructed using Maximum Likelihood (ML) due to its phylogenetic consistency and efficiency (Yang and Rannala, 2012). In addition, ML performs well in situations where phylogenies contain heterogeneous branch lengths, which is relevant to our phylogenies as they spanned both intra and interspecific taxonomic levels (Susko, 2008). The phylogenies were built using PhyML v3.0 and the GTR substitution model with 500 bootstrap replicates (Huong et al., 2016).

Bootstrap support values of 70% or higher were considered well supported (Hillis and Bull, 1993). Fine-scale evolutionary relationships among closely related sub-groups of operon sequences were inferred by constructing un-rooted statistical parsimony networks (Templeton et al., 1992) as implemented in the software package TCS version 1.13 (Clement et al., 2000). Invariant sites were removed from the global alignment for this analysis.

## 3. Results and discussion

### 3.1. BLAST/global alignments, and genetic differences among isolates

Representing six species (*S. suis*, *S. agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pasteurianus*, *Streptococcus hyovaginalis,* and *Staphylococcus aureus*), the BLAST search produced 439 hits (local alignments), of which 426 were from NCBI databases and 13 from the *S. agalactiae* database. However, some hit alignments contained as few as one gene. Therefore, as a filtering step, we excluded hits with fewer than nine operon genes. After inspecting BLAST alignments from all genome sequences corresponding to the remaining BLAST hits, nine BLAST alignments were found to have the nisin operon sequence truncated. Truncation of the operon was due to gene content differences at either end of the operon (Fig. 1) resulting in termination of the BLAST alignment immediately prior to the genes at either end. For each of the isolates involved, the corresponding operon sequence including the discrepant gene sequences were extracted from the relevant genome sequence and added to a global alignment of operon sequences.

Another 14 BLAST alignments were truncated due to the presence of an insertion sequence within the operon. For each of these isolates, the relevant operon sequence and the absent section of the operon, including the insertion sequence, was extracted from the relevant genome sequence and added to the global alignment. In this case, three *S. agalaticae* isolates contained a transposase within the *nisB* gene. There were also 11 *S. suis* isolates that had a Tn916-like transposon (Santoro et al., 2014; Wozniak and Waldor, 2010), containing 18 genes, inserted between the *nisK* and *nisF* genes (Fig. 2). An alignment where these insertion sequences were removed was generated as well as an alignment of just the Tn916-like transposons to gain a broader perspective of the distribution among taxa (see discussion below in Section 3.3). Insertion of the transposon did not disrupt the *nisK* and *nisF* gene sequences and both *nisRK* and *nisFEG*, which now flank the transposon, possess separate promoter sequences (de Ruyter et al., 1996; Ra et al., 1996). Consequently, it is possible that the nisin operon is still functional in these isolates. Finally, an additional five BLAST alignments had low coverage due to portions of the operon being located on different contigs. For four of the alignments, the operon was distributed between two contigs, which were paired and added to the operon alignment. For the fifth alignment, the operon was distributed among three contigs, which were also added to the global alignment. Fragmentation of these contigs occurred at a putative transposase in bovine *S. agalactiae* isolates. The final operon alignment contained 147 distinct sequences (strains) (Supplementary Table 1). The average nucleotide pairwise identity (API) of the alignment was 99.2%.

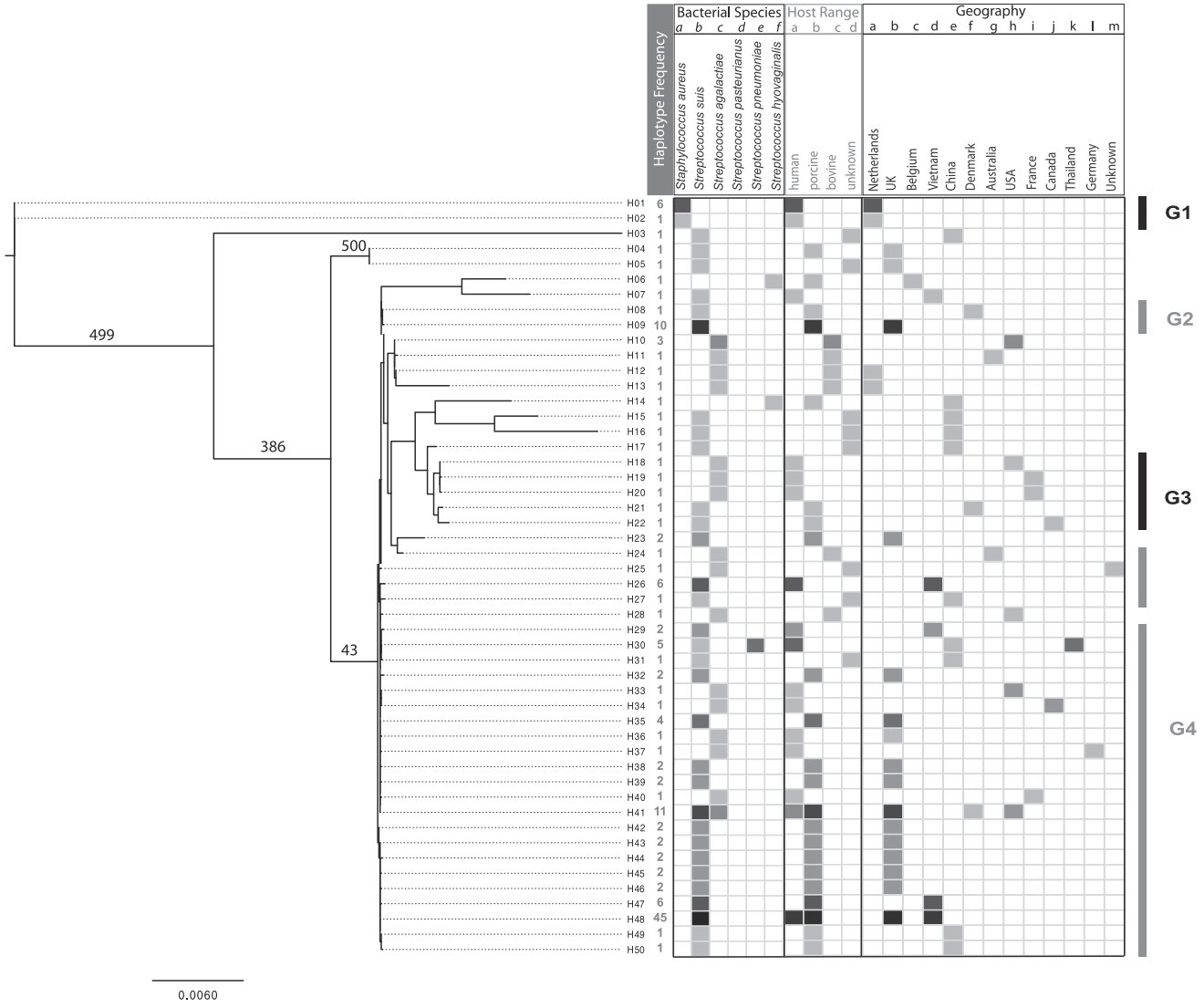


**Fig. 3.** Maximum likelihood phylogeny (500 bootstrap replicates) showing relationship among 50 nisin operon haplotypes. The matrix on the right shows the distribution of bacterial species containing the nisin operon, their originating host, and country of origin. Haplotypes are numbered H1-50. Numbers next to the haplotype ID represent the number of isolates within each haplotype. The shade of each rectangle is scaled based on the number of isolates in each haplotype, where black represents the highest frequency of isolates occurring in that category. Haplotype groups (G1-G4) (see Section 3.2) are highlighted with different shaded bars. G1 represents *S. aureus* isolates. G2 represents the isolates containing the Tn916-like transposon. Haplotypes in G3 and G4 produce two separate statistical parsimony networks (see Fig. 4 for G4 network). Note: haplotypes belonging to G4 are shown by two bars because haplotype 28 did not group in the statistical parsimony network.

### 3.2. Genetic exchange between species and hosts

The 147 operon sequences within the global operon alignment were sourced from six bacteria species originating from three different hosts (humans, bovine, and porcine). The most frequent bacterial species was *S. suis* (114/147, 77.55%) followed by *S. agalactiae* (20/147, 13.60%). The frequencies for the remaining species were as follows: *S. aureus* (7/147, 4.76%), *S. pneumoniae* (4/147, 2.72%), *S. pasteurianus* (1/147, 0.685%) and *S. hyovaginalis* (1/147, 0.685%). The most prominent host was porcine (88/147, 59.86%), followed by human (43/147, 29.25%) and then bovine (8/147, 5.445%). The host origins for seven *S. suis* and one *S. agalactiae* were unknown. Humans hosted the highest diversity of species, with *S. suis* being the most frequent, followed by *S. agalactiae* and lastly *S. aureus*. *S. pneumoniae* and *S. pasteurianus* were exclusively isolated from humans. One *S. hyovaginalis* isolate originated from porcine, which also most frequently hosted *S. suis*. The only isolates isolated from bovine were *S. agalactiae*. *S. suis* was historically regarded as a porcine pathogen. However, reports of human infection have continued to accumulate over the past 45 years and two epidemics were reported in China in 1998 and 2005 (Goyette-Desjardins et al., 2014). Consequently, *S. suis* appears to be an important emerging zoonotic pathogen. *S. agalactiae* is an important human pathogen, with the primary concern being neonatal infection, and has a particularly broad host range (e.g. cows, dogs, goats, camels, seals, dolphins, crocodiles, frogs, and fish). Despite its prominence in humans, it is also a major cause of bovine mastitis (the major production limiting disease in the dairy industry worldwide) (Keefe, 1997; Wilson et al., 1999), with bovine forming an important reservoir for the pathogen (Delannoy et al., 2013; Jorgensen et al., 2016).

Operon sequences for all 147 isolates were collapsed into 50 haplotypes. There were 31 singletons and the remaining 116 sequences were distributed among 19 different haplotypes (Fig. 3). For some haplotypes, the bacteria representing the haplotype were isolated from different species and hosts. More specifically, the operon sequences did not group according to the bacterial species they were obtained from. The operons were not monophyletic with regard to species and many

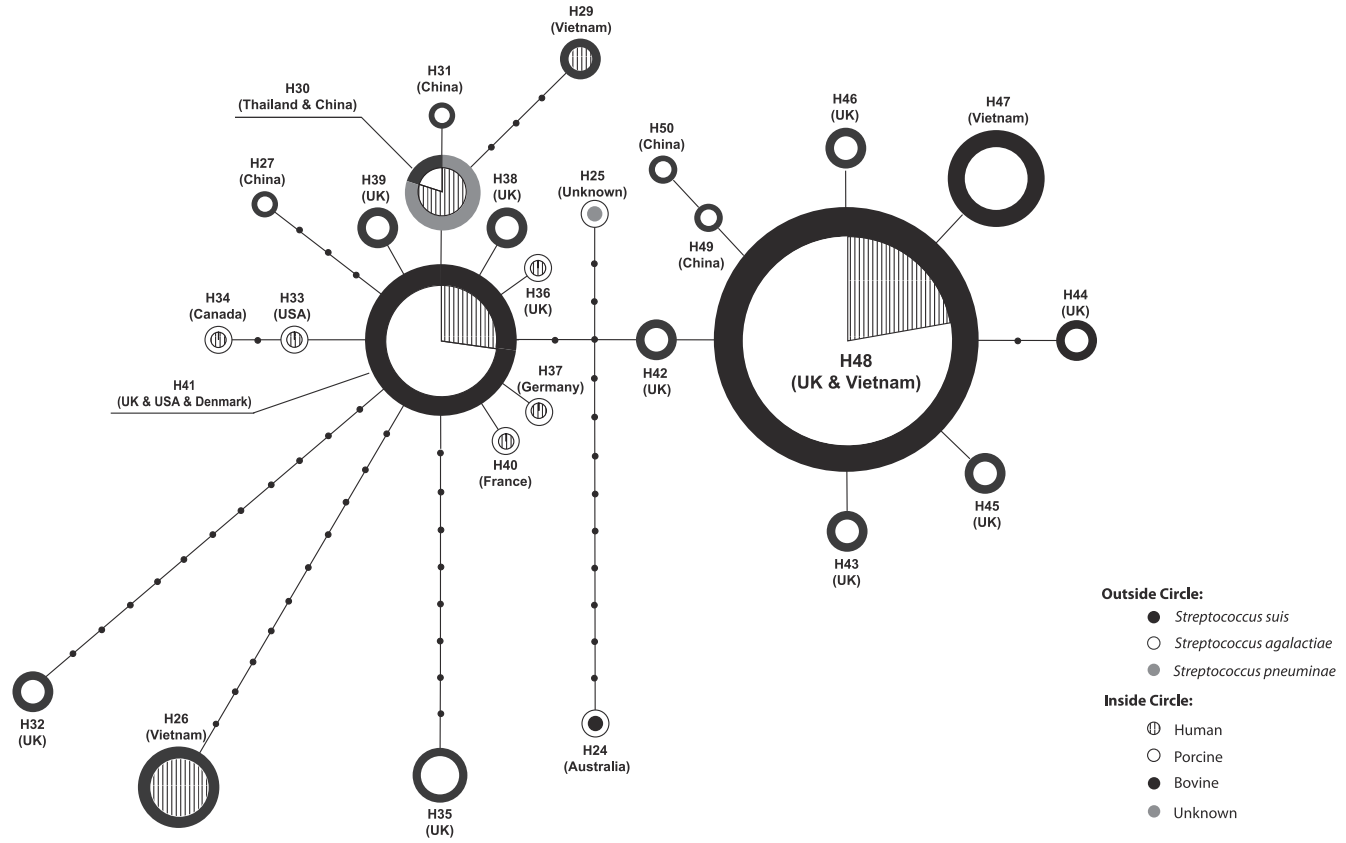


**Fig. 4.** Statistical parsimony network showing relationship among 26 nisin operon haplotypes (group G4, see Section 3.2). Haplotypes are shown by circles. Shade of the outside ring represents bacteria species from which the operon was obtained and the inside color/pattern represents the bacteria isolation source (host). Size of the circle is approximately proportional to the frequency of the haplotype. Lines connecting haplotypes represent mutational steps (SNPs) and small black circles represent hypothetical haplotypes that were not sampled. Haplotype IDs that can be cross-referenced to Fig. 3 are shown over haplotypes. Geographic location of isolation is shown in parentheses.

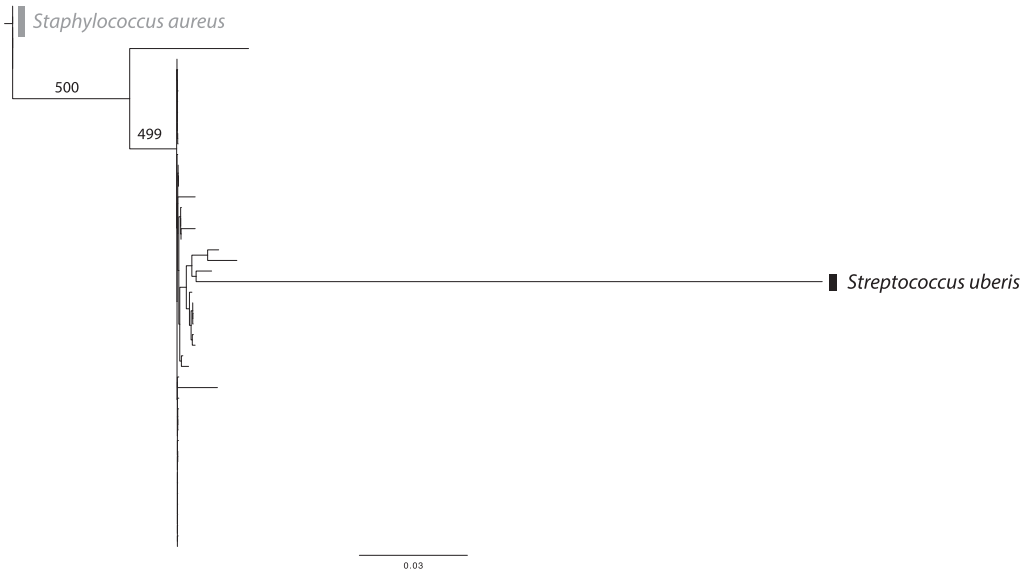


**Fig. 5.** Maximum likelihood phylogeny of the 50 nisin operon haplotypes with the addition of the nisin operon from *S. uberis*. The grey bar represents the two haplotypes of *Staphylococcus aureus*. The black bar represents the operon from *S. uberis*. The remaining tree branches represent operons distributed in the other *Streptococcus* isolates (details see Fig. 3). Bootstrap support values are shown over branches.

sequences grouped very closely or were identical to sequences from distantly related species. A likely explanation for such a phylogenetic pattern is lateral gene transfer, which in this case seems very plausible given that previous work of ours has shown the operon to be carried within an integrative conjugation element (ICE) (Richards et al., 2011). A specific example was haplotype 41 (H41, Fig. 3), representing 11 operon sequences, three of which were from human *S. agalactiae* and eight from porcine *S. suis*. Given that these sequences were 100% identical and that the operon is approximately 13kbp, this would suggest a relatively recent exchange between these species and hosts.

To gain a more fine-scale understanding of the evolutionary relationship among the haplotypes, a statistical parsimony analysis was performed using all operon sequences. The analysis joined two groups of haplotypes (G3 and G4) (Fig. 3) into two separate contiguous

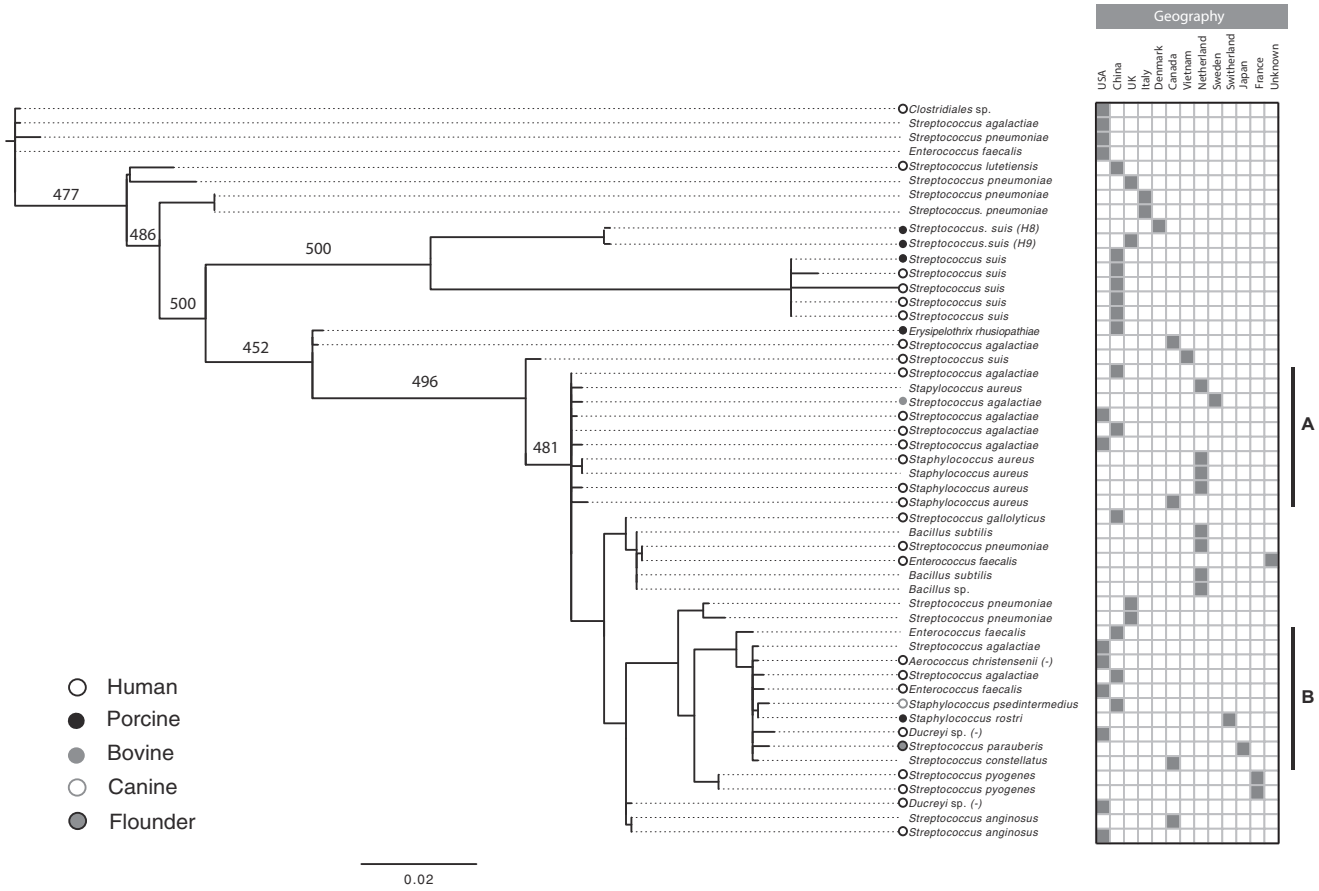


**Fig. 6.** Maximum likelihood phylogeny showing the relationship among Tn916–like transposon sequences. Different hosts are indicated with different shaded circles. Grey squares within the matrix show the geographic distribution of isolates. Groups A and B that have a close evolutionary relationship (see Section 3.3) are indicated by grey bars. Bootstrap support values are shown over branches.

networks. The largest of these groups (G4) contained 26 haplotypes and the network is shown in Fig. 4. Full operon sequences represented by these haplotypes had an API of 99.9%. The group contained 92 *S. suis* (human = 18; porcine = 71; unknown = 3), ten *S. agalactiae* (human = 8; bovine = 1; unknown = 1), and four *S. pneumoniae* (human = 4). The network again showed sequences to not group by species, with *S. agalactiae*, *S. suis*, and *S. pneumonia* frequently separated by only one SNP. The *S. pneumonia* haplotype was identical to one haplotype from *S. suis*. These haplotypes were isolated from both humans and pigs suggesting multiple cases of transmission between these hosts. Despite the virtually identical nature of these haplotypes, they were isolated from regions all over the world (Thailand, China, Germany, Denmark, France, UK, USA, and Canada), strongly suggesting that transmission is not restricted by geographic distance. This finding is concordant with the recent comparative genomic study of Weinert et al. (2015) that showed little geographical clustering of different *S. suis* subpopulations. Furthermore, they also showed that *S. suis* undergoes high rates of recombination, which would allow for LGT of the nisin operon. Group 3 (G3) contained three *S. agalactiae* haplotypes from humans and two *S. suis* haplotypes from porcine. However, unlike the network for G4, haplotypes in the G3 network did group by species (Supplementary Fig. S1).

Numerous typing studies have previously highlighted the possibility of transmission of pathogenic bacteria between humans and livestock (e.g. bovine, porcine, and poultry) (Dumke et al., 2015; Huijsdens et al., 2006; Ocepek et al., 2005; Pelkonen et al., 2013; Schultsz et al., 2012). Here we utilized a high-resolution molecular marker to provide evidence of transmission between humans and pigs. In particular, we provide strong evidence for transmission involving *S. suis*, further supporting this species as an emerging human pathogen (Weinert et al., 2015). Our results also confirm the possibility of transmission that was suggested by numerous sequence typing studies that showed the occurrence of the same sequence type in both humans and pigs in Asia, Europe, and North America (Goyette-Desjardins et al., 2014). Numerous disease outbreaks have been observed where animals created new pathogen reservoirs that can lead to human infection (Fey et al., 2000; Gaede et al., 2008; Huong et al., 2016; Jones et al., 2013), and our findings highlight the potential for pigs to act as reservoirs for the evolution of virulent strains that can subsequently spill over into the human population. Our approach, however, cannot confirm directionality, and consequently, the reverse should also be considered – humans acting as reservoirs (reverse zoonosis). Indeed, a recent literature review described 56 studies reporting possible transmission of multiple pathogens (bacteria, fungal, viral) from humans into livestock, wildlife, and companion animals (Klous et al., 2016; Messenger et al., 2014).

A third haplotype group, designated G1 in Fig. 3, contained nisin operon sequences from *Staphylococcus aureus*. These sequences did not group with those of the *Streptococcus* genus. However, this was solely due to the difference in the gene at the 3′ end between the two genera (*S. aureus* possessed a permease instead of nisI). *nisI* imparts nisin immunity, yet there may be some immunity function pertaining to the permease (Attia et al., 2010; Biswas and Biswas, 2013). Once this gene was removed, the operon was 100% identical with H42 (two porcine *S. suis*). Although the directionality of transmission is unknown, this high sequence identity might suggest a very recent exchange of the operon between the two genera and an even more rapid adaptation due to subsequent gene replacement. From the perspective of *S. aureus*, in combination with point mutations and differential gene expression, the

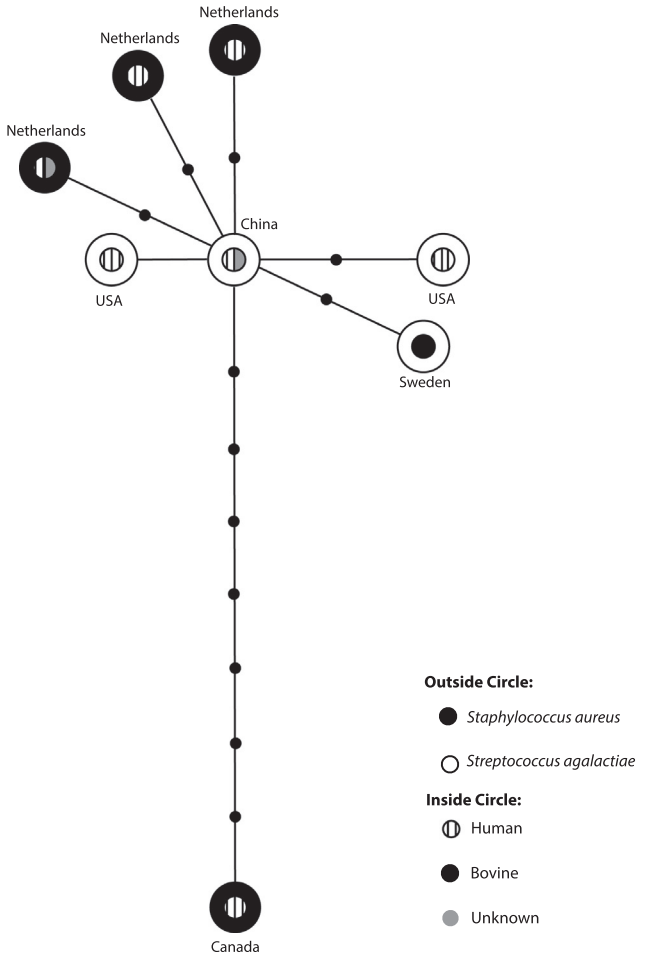


**Fig. 7.** Statistical parsimony network showing relationship among eight Tn916-like transposon haplotypes (group A, see Section 3.3). Haplotypes are shown by circles. Color of the outside ring represents bacteria species from which the transposon was obtained and the inside color/pattern represents the bacteria isolation source (host). Lines connecting haplotypes represent mutational steps (SNPs) and small black circles represent hypothetical haplotypes that were not sampled. Geographic location of isolation is shown next to each haplotype.

rapid acquisition of genes were the likely evolutionary mechanisms responsible for the evolution and rapid spread of the highly transmissible community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) (Thurlow et al., 2012).

Upon further review of the BLAST hits, we found that the operon sequence from *S. uberis* to have a relatively low sequence identity with the other operon sequences (88%). To gain a broader perspective of the evolution of the nisin operon, an additional phylogenetic analysis was performed including the nisin U operon from *S. uberis* (Fig. 5). The resulting phylogeny showed the *S. uberis* operon to be highly divergent from all of the other *Streptococci* operons. Indeed, it was more divergent from the other *Streptococcus* species than the *S. aureus* sequences were. A possible contributor to this observation is that *S. uberis* is a strict animal pathogen. Consequently, some of the observed nucleotide differences may reflect the fact that *S. uberis* has not adapted to the human host. For example, this restricted host range might allow for increased nucleotide diversification when compared to those taxa more able to cross the human-animal boundary. More specifically, frequent exchange of genetic material among species with a shared human environment would likely homogenize allele frequencies among those taxa yet result in diversification for taxa less able to exchange material. Similarly, MRSA showed a decreased capacity for human colonization after a host jump from humans to livestock (Price et al., 2012).

### 3.3. Tn916-like transposon

A fourth and last haplotype group, designated G2 in Fig. 3, contained two haplotypes (H08 [n = 1] and H09 [n = 10]), isolated from porcine *S. suis*, for which a Tn916-like transposon had been inserted between the *nisK* and *nisF* genes (Fig. 2). The transposon contained 18 genes and recent studies have shown it to exhibit the transposable properties of an ICE (Mikalsen et al., 2015; Mingoia et al., 2013). The two transposon sequences differed by two SNPs. The entire transposon sequence (belonging to H09) was searched against the nt/nr database at NCBI using BLASTn (E-value = 1e−5) to determine the distribution among different taxa. Forty-nine hits with 100% coverage and 99.2% to 99.6% sequence identity were obtained. The corresponding 49 transposon sequences plus the two from H08 and H09 were globally aligned and showed API of 99.7%. This alignment contained 30 different species spanning five different genera, including Gram-positive and Gram-negative bacteria (Supplementary Table 1). Similar to our results for the nisin operon, the Tn-916 family transposons have been found in multiple genera including *Streptococcus, Staphylococcus, Enterococcus* and *Bacilli* (Mikalsen et al., 2015; Pinto et al., 2014; Shimoji et al., 1998; Ye et al., 2008) and a maximum likelihood phylogeny of the 51 transposons (Fig. 6) showed that the transposon sequences did not group by species, with highly divergent species often grouping together, confirming a lateral pattern of inheritance. The scope of LGT for the transposon appears extensive as both Gram-positive and Gram-negative host bacteria grouped closely in the phylogeny, with sequences showing 99.6% API in the global alignment.

Again, to gain a more fine-scale understanding of the evolutionary relationship for the transposon, a statistical parsimony analysis was performed using all transposon sequences. The analysis produced two contiguous networks (A and B) that likely reflect transmission between animal and human hosts (Figs. 7 and 8). Sequences forming these networks are highlighted in the phylogeny (Fig. 6). Network A (99.98% API) included sequences from *S. agalactiae* and *S. aureus* isolated from both human and bovine hosts. Network B (99.97%) contained sequences from six Gram positive bacteria: *S. agalactiae* (human), *Streptococcus constellatus* (unknown), *Staphyloccocus psedintermedius* (canine), *Staphyloccocus rostri* (porcine), *Streptococcus parauberis* (flounder), *Enterococcus faecalis* (human), and two Gram negative bacteria: *Aerococcus christensenii* (human), and *Ducreyi* sp. (human). Within network B, seven sequences were separated by two or three SNPs. These sequences represented six species and three hosts (human, porcine, and canine). Of note is the canine host, highlighting possible transmission between humans, livestock, and companion animals. Consideration of this transmission route is warranted given the intimate association between humans and canine companion animals. For example, within the US alone, a 2017 survey estimated there to be approximately 89.7 million dogs as pets (APPA, 2017). These sequences also represented four countries: China, USA, Canada, and Switzerland, again suggesting that transmission was not restricted by geography. Within network A, all but one sequence was separated by one or two SNPs, with these sequences representing four countries: China, USA, Netherlands and Sweden, once more suggesting that transmission was not restricted by geography. Importantly, the transposon contained the *tetM* gene, which encodes the tetracycline resistance protein. Possession of *tetM* has been correlated with human outbreaks of *S. suis* in China (Ye et al., 2008) and its acquisition by *S. agalactiae* may have led to the fixation of clonal lineages well adapted to humans (in particular neonates) (DaCunha et al., 2014). Our phylogenetic analysis shows how readily this gene can be transferred among diverse species and environments.

## 4. Conclusion

Here we provide evidence for bacterial transmission between humans, livestock, and a companion animal. This transmission appears to occur over large geographic distances. Both the globalization of the

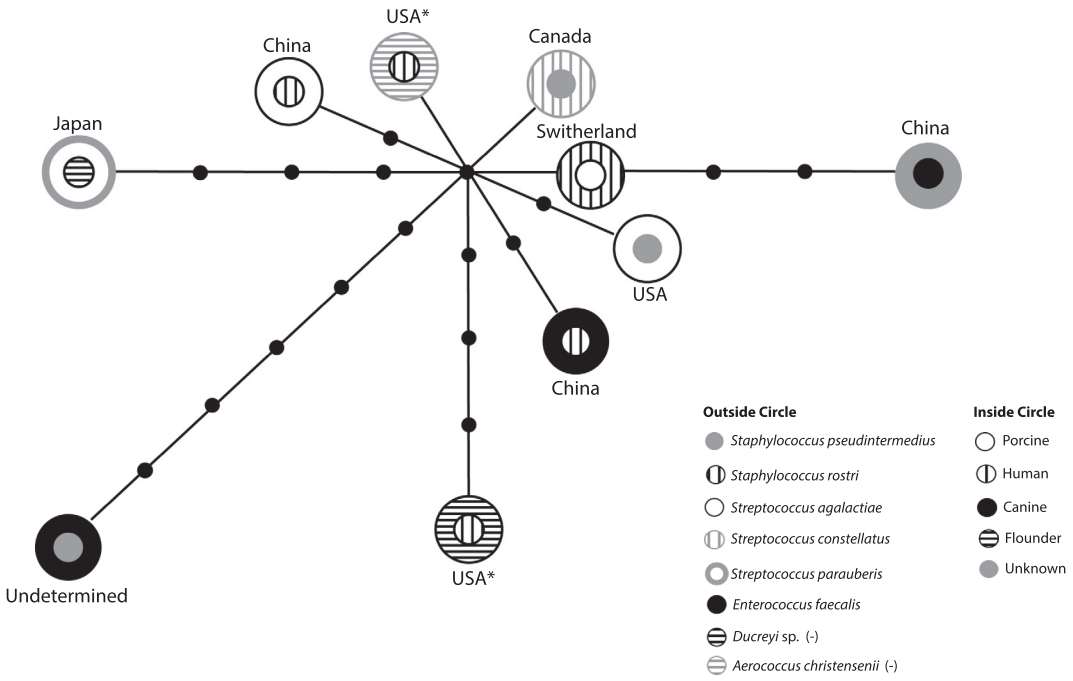


**Fig. 8.** Statistical parsimony network showing relationship among ten Tn916-like transposon haplotypes (group B, see Section 3.3). Haplotypes are shown by colored circles. Color pattern of the outside ring represents bacteria species from which the transposon was obtained and the inside color represents the bacteria isolation source (host). Lines connecting haplotypes represent mutational steps (SNPs) and small black circles represent hypothetical haplotypes that were not sampled. Geographic location of isolation is shown next to each haplotype. Asterisk shows Gram-negative species.

food industry and an increasingly mobile and expanding human population are likely contributing factors. For example, in 2015, the International Civil Aviation Organization (ICAO) reported 3.5 billion air passengers for that year. Furthermore, passenger kilometers have increased dramatically in the past 20 years: from approximately two trillion in 1996 to seven trillion in 2016. This trend is likely to increase with passenger kilometers projected to be 12 trillion by 2030. However, animals and animal products are also being transported extensively (Brown, 2004). As a result, it is now possible for a pathogen to be transported across the globe in less than 24 h (Wilson, 2003). A specific example of how quickly a pathogen can spread globally involved the influenza H1N1 virus. In 2009, this virus spread the globe and adapted to the porcine host within two months (Song et al., 2010). However, in addition to concerns regarding zoonosis, our findings also highlight the potential threat to livestock worldwide due to reverse zoonosis. Consequently, as the interaction between humans and animals becomes more intertwined, the One Health initiative becomes increasingly relevant. This global initiative aims to achieve the best health for people and animals through improved collaborative disease surveillance and response strategies that are attained through the integration of multiple disciplines including human medicine, veterinary medicine, and environmental and biological sciences.

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Conflicts of interest.

None.

## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ympev.2018.03.034.

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