Survey of the *bp/tee* genes from clinical group A streptococcus isolates in New Zealand – implications for vaccine development

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Group A streptococcus (GAS) is responsible for a wide range of diseases ranging from superficial infections, such as pharyngitis and impetigo, to life-threatening diseases, such as toxic shock syndrome and acute rheumatic fever (ARF). GAS pili are hair-like extensions protruding from the cell surface and consist of highly immunogenic structural proteins: the backbone pilin (BP) and one or two accessory pilins (AP1 and AP2). The protease-resistant BP builds the pilus shaft and has been recognized as the T-antigen, which forms the basis of a major serological typing scheme that is often used as a supplement to M typing. A previous sequence analysis of the *bp* gene (*tee* gene) in 39 GAS isolates revealed 15 different *bp/tee* types. In this study, we sequenced the *bp/tee* gene from 100 GAS isolates obtained from patients with pharyngitis, ARF or invasive disease in New Zealand. We found 20 new *bp/tee* alleles and four new *bp/tee* types/subtypes. No association between *bp/tee* type and clinical outcome was observed. We confirmed earlier reports that the *emm* type and *tee* type are associated strongly, but we also found exceptions, where multiple *tee* types could be found in certain M/*emm* type strains, such as M/*emm*89. We also reported, for the first time, the existence of a chimeric *bp/tee* allele, which was assigned into a new subclade (*bp/tee*3.1). A strong sequence conservation of the *bp* gene was observed within the individual *bp/tee* types/subtypes (>97% sequence identity), as well as between historical and contemporary New Zealand and international GAS strains. This temporal and geographical sequence stability provided further evidence for the potential use of the BP/T-antigen as a vaccine target.

INTRODUCTION

*Streptococcus pyogenes* [group A streptococcus (GAS)] is a major human pathogen. GAS causes a number of diseases ranging from non-invasive skin and soft tissue infections, such as impetigo and pharyngitis, to severe invasive disease, such as necrotizing fasciitis and toxic shock syndrome. In addition, GAS infection can result in non-suppurative sequelae, such as acute rheumatic fever (ARF) and post-streptococcal glomerulonephritis (Walker *et al.*, 2014).

Historically, GAS was classified using two major serological markers – the M- and T-Lancefield antigens, which have subsequently been shown to correspond to the fibrillar M protein and components of the GAS pili, respectively (Fischetti, 1989; Mora *et al.*, 2005). The GAS pilus consists of multiple copies of the backbone protein (BP), which polymerizes to form the pilus fibre, and one or two accessory proteins (APs); AP1 is located at the pilus tip and serves as an adhesin, and AP2 is located at the pilus base of most pilus types and functions as an adaptor protein for sortase-mediated cell wall anchoring. GAS pilin genes, together with genes encoding pilus-assembly sortases, are found in a single operon within the FCT (fibronection-binding, collagen-binding, T-antigen) region (Kröller *et al.*, 2010; Kratovac *et al.*, 2007; Kreikemeyer *et al.*, 2011). So far,
seven FCT regions have been characterized by sequence analysis of reference strains (FCT1–6 and 9), with each major FCT region being distinguishable by the size and organization of genes present. An additional two FCT regions (FCT7 and FCT8) have been identified as single-gene variants of FCT4 by PCR (Falugi et al., 2008; Köller et al., 2010). All GAS isolates analysed to date carry one of the nine FCT regions, and there is a strong association between the FCT region and M/emm type. The FCT region, with its complement of pilins and extracellular matrix binding proteins, has been proposed as a determining factor in tissue tropism and biofilm formation (Abbot et al., 2007; Köller et al., 2010; Lizano et al., 2007).

With the development of fast and cost-effective DNA-sequencing methods, M serotyping has been replaced by emm gene sequence typing that involves sequencing a small variable portion at the 5′ end of the complete emm gene, which encodes the M protein (Beall et al., 1996). Using this method, >200 emm types have been identified (Steer et al., 2009). After the more recent identification of the BP as the T-antigen (Mora et al., 2005), T serotyping can now also be replaced by bp/tee typing. Falugi et al. (2008) sequenced the complete bp/tee gene of 39 GAS isolates representing 23 different emm types. The bp/tee genes were classified into 15 clusters with sequence identity within each cluster of >90%. The majority of isolates were found to harbour bp/tee alleles that are highly conserved (>50% identity), which are encoded in the FCT3/4/7/8 region (17 of the 23 M/emm types analysed). The emm1 isolates were found to harbour bp/tee alleles encoded by the FCT2 region that share 36–40% identity with bp/tee alleles in the FCT3/4/7/8 region. In contrast, the bp/tee genes located in the FCT1/5/6/9 regions differ significantly, with <30% identity between each other and the FCT2/3/4/7/8-associated bp/tee genes. These FCT regions are rare and were only found in one or two M/emm types. For example, FCT1 was found in M6 and M23 strains, whilst FCT5 was found in M4 strains only.

The phylogenetic clustering of bp/tee alleles with respect to FCT region and the low sequence identity between the FCT1/5/6/9 regions compared with the FCT2/3/4/7/8 regions reflect the domain architecture of the BP/T-antigens. Thus far, two BP/T-antigens have been crystallized and the protein structures show a modular architecture comprising two or three immunoglobulin (Ig)-like domains. The M/emm1 BP/T-antigen (FCT2) possesses two domains and, based on protein sequence alignments, it can be assumed that all FCT2/3/4/7/8 BP/T-antigens share this two-domain architecture (Kang et al., 2007). The recently solved structure of the M/emm6 BP/T-antigen (FCT1) comprises three Ig-like domains (Young et al., 2014), and sequence alignments suggest that FCT6- and FCT9-encoded BP/T-antigens are also three-domain proteins, whereas FCT5/emm4 BP/T-antigens are believed to consist of four Ig-like domains (Spraggon et al., 2010).

Overall, the BP/T-antigen shows significantly less antigenic variation compared with the M protein and this is reflected in the lower number of T serotypes (21 T serotypes compared with ~100 M serotypes). The BP/T-antigen is a potential GAS vaccine candidate due to its high immunogenicity and protective properties in murine infection models (Mora et al., 2005). It has been hypothesized that compared with an M-protein-based vaccine, a BP/T-antigen-based vaccine would require fewer antigens to achieve comparable strain coverage (Falugi et al., 2008). The rates of ARF in Maori and Pacific children in New Zealand are amongst the highest in the world (Walker et al., 2014), and developing a GAS vaccine is the most practical solution to reducing disease burden in the longer term (Moreland et al., 2014). To further evaluate the BP/T-antigen as a suitable vaccine candidate in New Zealand, we undertook a broad sequence survey of the bp/tee alleles. The bp/tee genes from 100 clinical isolates, representing the most common pathogenic M/emm types found in New Zealand, were sequenced and compared with 34 bp/tee gene sequences from international isolates to evaluate the diversity of bp/tee genes in different geographical settings. Our New Zealand strain collection includes 25 historical isolates enabling temporal variations in the bp/tee gene to be assessed. As the isolates were derived from a broad spectrum of GAS disease, we also explored whether there was any association between bp/tee alleles and clinical manifestation. As in previous studies (Kratovac et al., 2007), we found a strong association between the M/emm type and bp/tee gene, with GAS strains belonging to the same M/emm type usually carrying the same bp/tee gene, but with several notable exceptions.

METHODS

We obtained 100 historical and contemporary GAS clinical isolates from the Institute of Environmental Science & Research, Wellington, New Zealand and Wellington Hospital, Wellington, New Zealand. The 75 contemporary strains in the collection were isolated between 2010 and 2013, and selected by M/emm type using recent epidemiological data. This ensured the most common M/emm types currently associated with pharyngitis (Williamson et al., 2014), invasive disease (Safar et al., 2011) and ARF (D. Williamson and P. Carter, personal communication) in New Zealand were analysed. The 25 historical strains were isolated in New Zealand between 1985 and 1995, and were included for temporal comparison. For further information, refer to Table S1 (available in the online Supplementary Material). All contemporary isolates were emm typed and the historical isolates were either emm typed or M typed at the Institute of Environmental Science & Research.

To prepare genomic template, sedimented bacteria from 250 µl overnight cultures were resuspended in 100 µl sterile MQ water and heated at 95 °C for 10 min. After centrifugation at 15500 g for 1 min, the supernatant was removed and stored at −20 °C. Supernatants were used in PCR at a ratio of 1 µl per 50 µl reaction volume. The bp/tee gene was amplified from the genomic template using Taq DNA polymerase with primer pairs designed to anneal to conserved regions in the 5′ signal sequence and the 3′ region downstream of the sortase cleavage site, as follows: FCT3 and FCT4, forward 5′-GCTTTATTGATAACCTGTAC-3′; reverse 5′-TCTCA-ACTTATTAAAGGTCG-3′; FCT2, forward 5′-AACAAAGTAAAGTCCTCCAC-3′; reverse 5′-CTTACTATTACGGGACGACGAG-3′; PCR
cycling conditions were: one step of 94 °C for 4 min; 35 cycles of 94 °C for 30 s, 52 °C for 30 s and 72 °C for 4 min; one step at 72 °C for 5 min. Each sample was purified on a QIAquick PCR purification column (Qiagen), typically yielding 1–2 μg DNA. The PCR products were subject to Sanger sequencing at ~80 ng μl⁻¹ with the same primers as used for PCR amplification. In addition, 34 bp/tee sequences from international GAS strains were downloaded from the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/). The bp/tee genes of the three- and four-domain pilins from GenBank accession numbers EU725486 (M2), EU725507 (M75), EU725491 (M4), EU725494 (M6) and DQ106872 (M23) were used for comparison. Sequence manipulation, analysis, alignments, and reconstruction of phylogenetic trees were performed using Geneious software version 7 (Biomatters). Unrooted phylogenetic trees were produced by the neighbour-joining method and bootstrapped 500 times. For all comparisons, the DNA regions encoding the signal sequence (as defined by SignalP; Petersen et al., 2011) and all residues after the sortase signal [(V/I)PTG] were excluded from the nucleotide sequence during alignments. All sequences were submitted to GenBank and assigned accession codes KJ816940–KJ817040.

RESULTS AND DISCUSSION

Bp/tee typing of New Zealand GAS strains

A sequence survey of the bp/tee gene in clinical isolates representing the most common pathogenic M/emm types found in New Zealand was undertaken to further establish the bp/tee gene as an epidemiological marker and evaluate the BP/T-antigen as a suitable vaccine candidate. Our GAS collection comprised 100 strains with 35 different emm types (Table S1) with a focus on bp/tee genes found in the FCT3/4 regions, as these were previously identified as the most diverse in terms of number of associated emm types (Köller et al., 2010). The collection included 22 M/emm types that had never been bp/tee typed before (Fig. 1, Table S2).

It has been reported that the FCT7 region, which lacks the ap1 gene, carries a bp/tee gene that is related to the bp/tee genes found in the FCT3 and FCT4 regions (Falugi et al., 2008). Indeed, we found that the FCT3/4 primers also amplified the bp/tee gene sequence of FCT7-associated M/emm99 samples and these clustered well inside the FCT3/4 clade (Fig. 1). The M/emm99 bp/tee gene was most closely related to FCT3-associated bp/tee genes from emm74 and emm55 strains, supporting the hypothesis that FCT7 is a derivative of FCT3 or FCT4 that has lost the ap1 gene (Kratovac et al., 2007). We also included five isolates of type M/emm1, which exclusively harbour the FCT2 region. The bp/tee gene of FCT2 was closely related to bp/tee genes of the FCT3, FCT4 and FCT7 regions (45–50 % nucleotide sequence pairwise identity) and the corresponding BP shared a two-domain protein structure, in contrast to the three- or four-domain structure of BP encoded in FCT1, FCT5, FCT6 and FCT9 (Falugi et al., 2008; Köller et al., 2010; Kreikemeyer et al., 2011).

The 100 bp/tee gene sequences from New Zealand and 34 international bp/tee sequences downloaded from the NCBI database were aligned. Phylogenetic trees of all aligned sequences showed 13 major clades, of which three could be further divided into two subclades, with sequence identities >97 % within each clade or subclade (Figs 1 and S1). The five clades represented by bp/tee genes located in the FCT1, FCT5, FCT6 and FCT9 regions were not included in the survey, but are shown in Fig. 1 for completeness. For naming newly identified bp/tee types, a bp/tee designation was assigned that corresponded with M/emm type where known and arbitrary numbers thereafter (Fig. 1, Table S1). This naming scheme is compatible with a trinary pilus system proposed by Falugi et al. (2008), where they assigned sequence types without consideration of serotypes. A cut-off of <65 % sequence identity was used to define a new type and <95 % identity for a new subtype. Using these values, three new bp/tee types and one new bp/tee subtype were identified, which were named bp/tee3.1, 7, 10 and 14. In addition, 20 new bp/tee alleles were found, increasing the total number of unique bp/tee alleles to 42, including 37 two-domain pilins from FCT2/3/4/7/8 and five from FCT1/5/6/9. (Figs 1 and S1). The bp/tee alleles derived from strains with the same emm type were usually identical or very similar (<8 nt difference), although there were some exceptions. There were three M/emm type strains that were associated with multiple bp/tee types. The bp/tee genes from M/emm89 strains clustered into three separate clades (bp/tee10, 11 and 14), with only one of these containing sequences similar to those from previously published studies, bp/tee11 (100 % nucleotide identity with GenBank accession number EU725511 from Falugi et al., 2008). M/emm44 samples from international, historical and contemporary collections harboured bp/tee that clustered as bp/tee5, 11 and 12, respectively. Similarly, two M/emm5 isolates carried different bp/tee alleles that shared only 55 % identity and therefore represented different bp/tee types. The international isolate M5_Manfredo carried the bp/tee5 type and the historical New Zealand isolate M5_085 harboured bp/tee9 (Fig. S1). In cases where bp/tee and emm type were not congruent, the bp/tee sequences were very similar or identical (>97 %) with other bp/tee alleles, indicating horizontal gene transfer between different M/emm types. In addition, a chimeric bp/tee allele was observed in the contemporary emm78 isolate 171, where the 5’ and 3’ regions aligned to bp/tee sequences found in bp/tee5 and bp/tee3, respectively (Fig. 2). The new bp/tee type was assigned into a new subclade (bp/tee3.1). This is the first reported case of a recombination between two different bp/tee alleles from different GAS strains.

In a recent study, Sanderson-Smith et al. (2014) introduced a new GAS typing system that combined functional classification of M protein and emm sequence, which allowed grouping of 175 emm types into two clades, two subclades and 48 emm clusters. The bp/tee phylogeny presented in this work shows no appreciable similarity to this emm phylogeny. For example, emm103, 49, 9, 44 and 113 strains were found in cluster E3, whereas when grouped by bp/tee gene sequence, these emm types clustered as bp/tee8, 18.2, 9, 5/11/12 and 12, respectively (Fig. S1).
**Fig. 1.** Phylogenetic tree of 37 full-length unique *bp/tee* gene sequences encoding two-domain proteins, defined into 13 clades and six subclades. The *bp/tee* genes of three- and four-domain pilins are also included to show the full range of known pilin diversity, bringing the total unique *bp/tee* allele count to 42 in 18 clades and six subclades. Assigned *bp/tee* types are correlated with existing T serotypes where sequence serology links have been established previously. Novel clades were assigned unused consecutive type numbers. Branch tip numbers indicate the M/emm types that cluster in each clade. Underlined M/emm types highlight the occurrence of multiple *bp/tee* types within a single M/emm type; those in bold had not previously been *bp/tee* typed. Previously unknown *bp/tee* types or subtypes are shown in blue. Branch number labels indicate the bootstrap consensus percentage. FCT types are also indicated; these were not determined empirically and were assigned to new pilin sequences by association with known FCT3/4/7 *bp/tee* sequences.

**Fig. 2.** Evidence for domain recombination of the *bp/tee* gene in isolate *emm78_171*. Shown here is an amino acid translation alignment of *emm78_171* (top) with *emm3_219* and *emm82_153*. Residues identical to the reference sequence (*emm78_171*) are shaded grey; residues not identical to the reference sequence are black. The first 130 residues of *emm78_171*, constituting the majority of the N-terminal domain, are identical to isolate *emm3_219*, with the remaining 160 C-terminal residues showing >97% identity to isolate *emm83_153*. 

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Comparison of historical and contemporary New Zealand and international bp/tee alleles

To assess if there were any temporal changes in bp/tee genes within the same geographical region, comparisons were made between historical (1990s) and contemporary samples (2010s) from New Zealand. Very little variation was observed between historical and contemporary bp/tee sequence (≤2 nt). Furthermore, comparison with international strains showed this high sequence conservation extended beyond New Zealand, with very little variation between New Zealand and international strains (≤6 nt difference). All three categories (historical, contemporary and international) clustered freely together in the pruned bp/tee phylogeny shown in Fig. 3, with only occasional single nucleotide polymorphisms separating the tightly clustered alleles in each clade [Fig. 3; historical (n=25), contemporary (n=75) and international (n=34)]. For example, the five isolates belonging to the common pathogenic strain M/emm1 type showed no bp/tee sequence variation between New Zealand and international strains, whilst all of bp/tee13, including international, historical and contemporary New Zealand strains with four distinct M/emm types (33, 41, 53 and 93), showed 100% conservation of the bp/tee gene (Figs 3 and S1).

The largest sequence variations were found in the bp/tee18.2 type, harboured by M/emm49 strains; the international strain NZ131 differed by 6 nt from the four New Zealand M/emm49 strains, including two non-synonymous mutations. However, the four New Zealand M/emm49 strains carried identical bp/tee18.2 alleles.

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**Fig. 3.** Comparison of bp/tee gene sequences in historical (strain numbers ≤100), contemporary (strain numbers ≥105) and international (shown with GenBank accession numbers or strain names) GAS isolates. Where bp/tee clusters with emm type, over time, locally and globally, there is very little divergence in bp/tee sequence. Two clusters exemplifying this trend: M/emm53 and M/emm89 (boxed). Where M/emm type and bp/tee type diverge, the bp/tee gene clusters closely with known bp/tee sequences found in other M/emm types, as shown by M/emm44 samples (indicated by arrows). Branch number labels indicate the bootstrap consensus percentage.
Particular focus was given to M/emm89 (25 strains) and M/emm12 (15 strains) as these are amongst the five most common M/emm types associated with pharyngitis and invasive disease in New Zealand (Safar et al., 2011; Williamson et al., 2014) (Fig. S1). M/emm12 strains showed 99% nucleotide identity in bp/tee sequences from all three categories (historical, contemporary and international) (Fig. 3). The most diverged M/emm12 bp/tee allele, M12_048, showed only 3 nt differences from all other M/emm12 bp/tee alleles, but showed 100% identity to a closely related bp/tee allele from M/emm22 strains. M/emm89 strains are notable in that they associated with three distinct bp/tee types (bp/tee11, 10 and 14), as discussed above. Where M/emm89 samples shared the same bp/tee type, identical bp/tee alleles were found in historical, contemporary or international strains (Fig. 3).

Phenotypic variation in BP/T-antigens

To examine phenotypic variation, the amino acid sequences of translated BP/T-antigen variants were analysed. Translated bp/tee gene sequences grouped into the same 18 clades and six subclades in a phylogenetic tree with >96% amino acid sequence identity within each clade or subclade (Fig. 1). Sequence alignments of two-domain bp/tee alleles showed 37 unique alleles corresponding to 34 translated BP/T-antigen variants (Fig. 4). Within all available FCT3/4/7 sequences, including those obtained from the NCBI, there were low numbers of highly conserved positions within BP/T-antigens: 63 of a mean of 314 aa residues (with gaps) (i.e. 20.6%). That figure drops to 48 (15.3%) when BP/T-antigens from FCT2 (M/emm1) are included. However, most substitutions were conservative with biochemical similarity, e.g. hydrophobic...
residues, such as valine, leucine and isoleucine, or aromatic residues, such as tyrosine, lysine and phenylalanine (BLOSUM62, pairwise identity 64%).

No variation in protein sequence was found amongst M/emm (FCT2) BP/T-antigens and these BP/T-antigens formed a clade separate from FCT3/4/7-associated BP/T-antigens, with a mean of 30% pairwise identity between M1 BP/T-antigens and FCT3/4/7 BP/T-antigens in a one-to-one alignment. This suggested that only a small number of conserved residues are required to maintain pilin structure and function. Variation appeared to be clustered on the BP/T-antigen with regions of conservation interspersed with regions variable in both length and sequence. Residues involved in pilus assembly and isopeptide formation were highly conserved in all FCT2/3/4/7-associated BP/T-antigens (Fig. 4).

An M/emm89 strain carries a bp/tee pseudogene

The New Zealand contemporary pharyngitis isolate emm89_221 carries a non-functional bp/tee allele (Fig. 5). The bp/tee gene has an 8 bp deletion that introduces a stop codon, which terminates translation just after the first Ig-like domain. This deletion results in loss of the C-terminal sortase C recognition domain and an asparagine residue critical for formation of the isopeptide bond characteristic of Gram-positive pilins (Kang et al., 2007). Although we have not carried out any phenotypic analysis, it is unlikely this isolate is able to form an intact pilus structure. It should be noted that serum from the patient from which this isolate was collected showed reactivity to another BP/T-antigen by ELISA (data not shown). Therefore, it might be possible that the emm89_221 isolate was a carrier strain not involved in the disease symptoms. To our knowledge, this is the only example for a defective bp/tee gene described in the literature.

**Association of bp/tee with clinical syndrome**

When clinical data for each sample strain were overlaid onto the bp/tee sequence clusters, no clear relationship was observed between bp/tee type and clinical syndrome (Table S2). All three disease types (ARF, pharyngitis or invasive disease) were found in most clades. One exception was clade 5, showing only pharyngitis cases, but this may be an artefact of sampling bias as the clinical isolates were selected on the basis of emm type. The lack of association is highlighted by M/emm89 samples; in both of the two major clades in which M/emm89 strains cluster (bp/tee10 and 11), an even distribution of clinical syndromes was evident. The lack of correlation between bp/tee gene sequence and clinical data suggested that the bp/tee type has limited effect on the clinical consequences of infection. This was further supported by our findings that bp/tee sequences form a different phylogenetic tree from the M/emm type (Fig. 1) (Sanderson-Smith et al., 2014) and the M/emm type has been shown to generally correlate with clinical outcomes (Bessen & Lizano, 2010). Falugi et al. (2008) noted that minor pilin genes ap1 and ap2 form phylogenies that are different from the bp/tee gene. It may be that these, or other genes encoded in the FCT regions, may have a dominant role in the specific consequences of infection over and above the bp/tee gene.

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**Fig. 5.** Alignment of the bp/tee allele from the truncated gene mutant emm89_221 with a functional emm89 allele. Arrows labelled ‘Iso’ annotate the residues that are used to form the isopeptide bond. Numbers at the start of each line denote base pair number, excluding signal sequences. The region shown covers the last 13 residues of the N-terminal domain, including the isopeptide bond-forming residue N152, which is missing from the mutant allele. The deletion also creates a frameshift, resulting in premature termination of protein translation.
BP/T-antigen as a potential vaccine candidate

The most clinically advanced GAS vaccine candidates are chimeric M-protein-based constructs (26- and 30-valent M protein vaccines) that incorporate the highly variable N-terminal peptide sequences from multiple M proteins (Dale et al., 2013; Steer et al., 2009). These vaccines have been designed to provide maximum coverage against European and North American GAS strains, and whilst cross-protection against non-vaccine strains has been observed (Dale et al., 2013), questions remain around the usefulness of multivalent M protein vaccines in settings with high strain diversity (Moreland et al., 2014). The BP/T-antigen has a much lower level of natural diversity, which may simplify the design of multivalent or chimeric vaccines that provide coverage against all major strains. This study has identified 18 known BP/T-antigen types with amino acid sequence identities of >96% within each type. This is comparable with the 21 known T serotypes originally identified by Lancefield. There are various reasons why there may be 21 T serotypes but only 18 BP/T-antigen types; the missing serotypes may be rare and therefore under-represented in clinical isolates or some of the six subtypes defined in Fig. 1 may represent different serotypes. An alternative, but less likely, explanation is that some T serotypes correspond to other parts of the pilus as certain T sera have also been shown to detect AP1 by Western blotting (Falugi et al., 2008). Regardless, the high sequence conservation within each BP/T-antigen type is advantageous for vaccine design.

CONCLUSIONS

In this study, 75 contemporary and 25 historical GAS isolates from patients with pharyngitis, invasive disease and ARF were bp/tee typed. Twenty new bp/tee alleles and four new tee types/subtypes were identified, bringing the total to 18 bp/tee types and six subtypes. Within each type or subtype, tee alleles share >97% sequence identity. We confirmed the previous observation that emm type and tee type are strongly associated, but with some notable exceptions, such as a chimeric bp/tee allele which was assigned into a new subclade (bp/tee3.1) and a bp/tee pseudogene encoding a truncated BP/T-antigen. In general, individual tee alleles are highly stable over time and geographical location, suggesting that the BP/T-antigen might be a suitable target as a vaccine candidate.

ACKNOWLEDGEMENTS

This work was supported by research grants from Lottery Health New Zealand and the Maurice Wilkins Centre for Biodiscovery, University of Auckland. N.M. is a New Zealand Heart Foundation Research Fellow.

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