FEMS Microbiology Reviews, 2025, **49**, fuaf058
DOI: 10.1093/femsre/fuaf058
Advance access publication date: 11 November 2025
Review Article

Global spread of *Streptococcus pyogenes* A genomics-supported narrative review

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Editor:: Oscar Kuipers

Abstract

Group A *Streptococcus* (GAS) has recently reemerged as a leading cause of both mild and severe invasive infections worldwide, with recent upsurges in invasive disease among children and adults. Notwithstanding a partial synchronicity with the COVID-19 pandemic, this rapid global dissemination of more virulent GAS lineages has been promptly detected, as well as the molecular shifts underlying the observed changes in clinical patterns. Whole-genome sequencing (WGS)-based genomic epidemiology allowed us to gain relevant insights into this upsurge as it was happening. This review integrates the canonical research publication-based approach with genomic data and metadata and identifies a subset of genomic clusters playing a major role in invasive GAS (iGAS) infections worldwide, which were named as *Global Pathogenic Lineages* (GPLs). The four GPLs broadly coincide with five sequence types (STs): GPL1 with ST28, GPL2 with ST15 and ST315, GPL3 with ST52, and GPL4 with ST39. While non-GPLs clusters maintain a baseline reservoir of antimicrobial-resistance and virulence genes, GPLs show varying but noteworthy resistance profiles and are frequent causes of iGAS. The integration of WGS into routine diagnostics procedures is a forthcoming improvement, aimed not only at informing tailored therapy and implementing infection control strategies, but also to perform continuous surveillance. Ongoing WGS in clinical microbiology, as a matter of fact, will provide unparalleled insights into lineage emergence, transmission dynamics, and the geographic clustering of virulence and resistance determinants.

Keywords: *Streptococcus pyogenes*; invasive infections; iGAS; genomic epidemiology; phylogenetics; virulence

Introduction

Streptococcus pyogenes (also known as Group A *Streptococcus* or GAS) is a globally significant human pathogen responsible for a spectrum of diseases ranging from mild pharyngitis and impetigo to severe invasive infections such as necrotizing fasciitis and streptococcal toxic shock syndrome (STSS) (1–3). Beyond acute manifestations, GAS can trigger serious postinfectious sequelae like acute rheumatic fever (ARF), rheumatic heart disease (RHD), and poststreptococcal glomerulonephritis (4–6).

Despite consistently susceptible to all beta-lactam antibiotics, owing to its capacity for asymptomatic colonization, rapid transmission, and periodic emergence of hypervirulent clones (7–9), GAS still poses a substantial public health challenge.

GAS has a significant impact on socially disadvantaged communities worldwide, especially in terms of postinfectious sequelae, due to poor housing conditions and limited access to medical care among other factors. LMICs are afflicted by ARF and RHD, which still represent important causes of morbidity and mortality amongst children and young adults (Carapetis et al. 2016, Sika-Paotonu et al. 2016). *Streptococcus pyogenes* earned renewed attention also in high-income countries due to recent upsurges of scarlet fever and, more recently, of invasive GAS (iGAS) infections. Epidemiologically, GAS infections exhibit notable age-related clinical patterns (Oppegaard et al. 2015, Tyrrell et al. 2018). Indeed, respiratory tract infections, particularly pharyngitis and scarlet fever, are predominantly seen in children. Conversely, nonrespiratory and invasive infections such as cellulitis, necrotizing fasciitis, and STSS are more frequently diagnosed in adults. iGAS infections, defined by the isolation of *S. pyogenes* from normally sterile sites (e.g. blood, cerebrospinal fluid, and soft tissues), can rapidly progress to life-threatening conditions including pneumonia, septic shock, and multiorgan failure, often necessitating urgent medical intervention and intensive care support (14–17). The SARS-CoV-2 pandemic temporarily disrupted the typical transmission patterns of GAS. Notably, the incidence of respiratory infections caused by *S. pyogenes* saw a decline in 2020, likely due to widespread public health interventions including lockdowns, the use of filtering face masks, and reduced social contact (18–20). However, as mitigation measures relaxed, infection rates rebounded to prepandemic levels by 2022, emphasizing the resilience and opportunistic nature of GAS transmission (21–23). Interestingly, the pandemic served as a natural experiment demonstrating the role of social behav-

Received 1 July 2025; **revised** 5 November 2025; **accepted** 5 November 2025

ior and population immunity in GAS transmission. Surveillance reports from Germany, the UK, and Australia showed shifts in seasonal dynamics, age distribution of cases, and even dominance of certain *emm* types in the post-COVID-19 period (24–27). These shifts also highlighted the potential impact of "immune debt," whereby reduced microbial exposure during COVID-19 pandemic waves may have led to increased susceptibility to bacterial infections following the relaxation of public health measures (Amarsy et al. 2023, Ujiie 2025).

Clinical manifestations, diagnosis and management

GAS remains one of the most versatile human pathogens, displaying a spectrum of clinical syndromes that range from common sore throat to life-threatening bloodstream infections.

Noninvasive *S. pyogenes* (non-iGAS) infections are frequently diagnosed in the pediatric population and may display a wide array of clinical manifestations. Among these, the streptococcal pharyngitis (commonly called "strep throat") is the most frequent manifestation: it accounts for up to 30% of sore throat presentations in children aged 5–15 years (30–34) and oftentimes is associated with fever and tonsillar exudates. GAS pharyngitis can be followed by scarlet fever, a systemic rash resulting from capillary damage caused by GAS exotoxins and characterized by erythematous "sandpaper" rash and strawberry tongue (Pardo and Perera 2025). Non-iGAS can also cause skin and soft-tissue infections such as cellulitis, erysipelas, localized abscesses, and impetigo, the latter involving the superficial skin and manifests as honey-crusted lesions and often cocirculating with scabies in tropical or overcrowded settings (Steer et al. 2009, Sekizuka et al. 2017, Williamson et al. 2023), further underscoring how GAS is able to invade superficial tissues, with pathogenesis driven by a repertoire of exotoxins and adhesins (Smith et al. 2005, Bhavsar 2024).

In contrast, iGAS infections are relatively rare but marked by a dramatic shift in disease severity. Necrotizing fasciitis is a fulminant destruction of fascia and subcutaneous tissue, with mortality rates up to 30% even under aggressive surgical and antibiotic interventions (Bruun et al. 2021, Merola et al. 2024). STSS is a condition caused by high loads of GAS superantigens (such as SpeA and SpeC), which trigger a cytokine storm (Emgård et al. 2019) leading to a systemic disease characterized by rapid-onset hypotension and multiorgan failure, with high risk of progression to septic shock (Leśnik et al. 2025). Moreover, STSS is often associated with deep soft-tissue foci, where microbial source control can be challenging to achieve (43–45).

Beta-lactam antibiotics remain the cornerstone of therapy: penicillin G, ampicillin, and third-generation cephalosporins reliably eradicate GAS targeting the universally conserved penicillin-binding proteins (Ikebe et al. 2005). Clindamycin is added in severe iGAS for its antitoxin effect and postantibiotic suppression of exotoxin production, though emerging clindamycin-resistant isolates (up to 45% in some regions) underscore the need for ongoing susceptibility testing (White and Siegrist 2021, Lapthorne et al. 2024). Supportive critical-care measures (i.e. fluid resuscitation, vasoactive agents, and mechanical ventilation) are lifesaving in STSS and necrotizing fasciitis, but definitive management often hinges on prompt surgical debridement (Steer et al. 2009). Research into rapid diagnostics and optimized treatment algorithms for GAS pharynx, skin, and soft tissue infections remains a high priority, especially in pediatric cohorts, with real-time genomic surveillance playing an increasingly pivotal role.

Timely identification of GAS infections is crucial to curb transmission in critical settings and to prevent major complications, such as progression to iGAS disease. For instance, streptococcal pharyngitis may mimic or overlap with viral respiratory tract infections. Symptoms such as sore throat, fever, and malaise are shared with influenza and mononucleosis, potentially delaying recognition of a mild GAS infection which, if not correctly treated, may eventually evolve into an invasive infection.

As of today, the laboratory-based gold standard for GAS identification is culture on sheep-blood agar where, after 18–24 h of incubation, GAS colonies appear as beta-hemolytic and bacitracin susceptible (Spellerberg and Brandt 2016). Rapid antigen detection tests (RADT) expedite results (~10 min) with >95% specificity but relatively low sensitivity (70%–94%), prompting reflex cultures for negative tests in high-risk patients.

Nucleic acid amplification tests, including integrated polymerase chain reaction (PCR) platforms or specific loop-mediated isothermal amplification tests, deliver ≥95% sensitivity and specificity within 2 h on multiple sample types, facilitating early antimicrobial stewardship interventions (52–54). Saliva-based quantitative polymerase chain reaction (qPCR) and isothermal amplification assays are under evaluation to improve patient comfort and sampling throughput (Nakanishi et al. 2011, Peachey et al. 2024).

Clinical-based prediction approaches, such as the Centor and McIsaac scores, coupled with point-of-care molecular tests, show promise but require further validation across age groups and epidemiological settings (Willis et al. 2020, Kanagasabai et al. 2024).

Clinical success prediction approaches can be implemented because of the GAS susceptibility to beta-lactam antibiotics, particularly penicillin, which remains the treatment of choice (Yu et al. 2023). The high prevalence in pharyngitis underpinned decades of successful empirical therapy in absence of routine susceptibility testing (Kanwal and Vaitla). Nonetheless, in recent years a rise in resistance to alternative agents has been observed with macrolide resistance rates climbing to 21%–50% in specific regions (Kebede et al. 2021, Gergova et al. 2024) and tetracycline resistance determinants likewise appear in 35%–40% of isolates from diverse regions (Kebede et al. 2021, Gergova et al. 2024). Moreover, albeit their clinical relevance still needs to be assessed, specific mutations in the PBP2x gene associated with reduced *in vitro* susceptibility to beta-lactams have been observed at a global level (Musser et al. 2020).

Antimicrobial stewardship best practices now emphasize confirmation of GAS infection and performance of antimicrobial susceptibility testing (AST) prior to initiating therapy as well, particularly in areas with elevated macrolide or tetracycline resistance and the CDC pediatric outpatient guidelines advise against empiric antibiotics without RADT or culture for most children (CDC 2025b https://www.cdc.gov/antibiotic-use/hcp/clinical-care/pediatric-outpatient.html).

In the rapidly evolving clinical microbiology context, NGS technologies are reshaping the landscape. Shotgun metagenomic sequencing directly from throat swabs or tissue biopsies can identify GAS alongside viral or fungal copathogens within 24–48 h, overcoming the bias of culture and targeted assays (Wensel et al. 2022, Zhao et al. 2024). Whole-genome sequencing (WGS) of isolates now routinely occurs in reference laboratories for strain typing, outbreak detection, and *in silico* AST, with turnaround times shrinking to 36 h thanks to benchtop NGS platforms (Dunne et al. 2012, Deurenberg et al. 2017). Although still too expensive, clinical metagenomic pipelines demonstrate diagnostic utility in culture-negative iGAS presentations, guiding targeted therapy when con-

ventional methods fail (Wensel et al. 2022). Analogously, 16S rRNA deep sequencing from complex and polymicrobial infections offers a rapid, culture-independent screen for GAS unaffected by prior antibiotic use and yielding species-level resolution within hours (Wensel et al. 2022).

Phenotype and genotype are two corresponding and complementary approaches to the study of microbiology and not contrasting entities and the context of antimicrobial resistance serves as a paradigmatic example. Canonic "phenotypic" and molecular "genomic" approaches complement and support each other. On one hand, *in vitro* susceptibility testing measures the efficacy of a drug–isolate combination, which serves as the base for clinical approaches. On the other hand, genome-based approaches allow the identification of molecular mechanisms underlying resistance, leading to defining the evolutionary trajectories of a bacterial species.

It is hence possible to forecast that the expanding use of NGS technologies, as of today mostly used for research purposes, will also have positive repercussions in clinical microbiology settings. As a matter of fact, it is reasonable to expect that by leveraging genomic datasets it will be possible to extract valuable information (e.g. lineage identification or virulence profiling) as routine laboratory tests and without the need of sequencing facilities.

The synthesis of phenotypic assays and genome-enabled diagnostics, hence, does not represent a mere technological advance but should be rather considered as an integrative approach in how we conceptualize pathogenesis, resistance, and transmission: beyond diagnostics, the power of genomics also lies in its ability to connect molecular variation with virulence and epidemiology.

Routine GAS sequencing will allow the microbiology community to highlight the presence of specific genetic patterns, analysing (and potentially ending before their time) localized outbreaks, detecting the diffusion of well-known lineages, and assessing the emergence of novel ones.

Genomics will thus not replace classical microbiology but, rather, it will be another tool in the hands of microbiology laboratory professionals. It will allow a deeper understanding of resistance, virulence, and spread by placing each isolate in a more complex and comprehensive evolutionary and mechanistic framework.

The following sections explore how these genomic insights illuminate the population structure, mobile elements, and global dissemination patterns of *S. pyogenes*.

Such comprehensive genomic surveillance will lead to the study of horizontal gene transfer (HGT) mechanisms which, as a major driver of genomic variability, represent key evolutionary pathways in determining the pathogenic potential of a strain.

HGT and genomic plasticity

HGT, genomic plasticity, and mobile genetic elements of *S. pyogenes*

Genomic variability among GAS strains is associated with homologous recombination, genomic rearrangement events, and with the presence of mobile genetic elements (MGEs) including prophages, GAS phage-like chromosomal islands, integrative and conjugative elements (ICEs), integrative and mobilizable elements, and plasmids (Bessen et al. 2015, Davies et al. 2019). Phylogenetic analysis of housekeeping genes showed that GAS undergoes chromosomal recombination events. Recombination frequency varies among different *emm*-types, with *emm1* being one of the least recombinogenic lineages (Kalia et al. 2002, Turner et al. 2017, Jespersen et al. 2020), however, the globally predominant *emm1* lineage is associated with homologous recombination around the NADase *nga* gene, the NADase inhibitor *ifs* gene, and the streptolysin O *slo* gene (Nasser et al. 2014). A subset of *S. pyogenes* genomes contains large-scale rearrangements in the form of X-shaped inversions, either symmetrical relative to the *oriC-ter* axis, or asymmetrical, resulting in imbalanced replichore sizes. The latter are mainly associated with the presence of insertion sequences and prophages in the *S. pyogenes* genome (Bao et al. 2016, Jespersen et al. 2024). HGT in *S. pyogenes* can occur via transformation (uptake of free DNA), transduction (DNA transfer mediated by phages), and conjugation (DNA transfer mediated by conjugative elements).

Natural transformation in *S. pyogenes* has been observed at very low frequencies mainly in streptococcal biofilm models, and involves the induction of *sigX* and *sigX*-dependent gene expression within the competence regulon (Mashburn-Warren et al. 2012).

Generalized transduction of streptomycin resistance was first reported in *S. pyogenes* in 1968 (Leonard et al. 1968). The lytic phage A25 and related elements exhibit high-efficiency transduction due to a nonstringent packaging mechanism (McCullor et al. 2018). Additionally, lysogenic phages, such as T12-like prophages, have been shown to mediate transduction, facilitating the transfer of antibiotic resistance genes (Hyder and Streitfeld 1978). Most *S. pyogenes* genomes are polylysogenic, which means they feature at least two prophage sequences integrated. The first genomes devoid of complete prophage sequences were first described in an *emm59* genome (Fittipaldi et al. 2012) and then in a subclade of *emm89* strains (Beres et al. 2016). Several lysogenic phages of *S. pyogenes* carry antibiotic resistance determinants (Brenciani et al. 2010, Iannelli et al. 2014). This represents a distinctive feature, as bacteriophages rarely carry antibiotic resistance genes (Enault et al. 2017), despite their recognized role in facilitating HGT. Lysogenic transfer of antibiotic resistance carrying phages can occur among different streptococcal species and is typically not associated to plaque production (Di Luca et al. 2010, Santoro et al. 2023).

Conjugation in *S. pyogenes* is well documented for ICEs and plasmids, with transfer occurring both within the species and to other streptococcal species.

Plasmids are relatively rare in GAS. Small plasmids of about 3 kb in length encoding a bacteriocin gene were characterized in M57 strains (Heng et al. 2004), while slightly larger plasmids (about 5 kb in length) coding for the macrolide resistance gene *erm*(T) were found in different *S. pyogenes* lineages (Woodbury et al. 2008) and are nearly identical to plasmids circulating in *Streptococcus agalactiae* (DiPersio et al. 2011). Conjugative plasmids of about 30 kb were isolated and characterized starting from the 1970s, the prototype being pSM19035, which harbors the *erm*(B) gene for macrolide, lincosamide, and streptogramin (MLS) resistance (Behnke et al. 1979). Notably, these plasmids appear to be absent in more recent genome sequences.

Virulence elements

In GAS, the expression of virulence genes is regulated by a complex network that includes at least 13 two-component systems and over 30 transcriptional regulators (Vega et al. 2022). The main role of these elements is to control and coordinate the response to internal and external signals (e.g. the current metabolic condition of the bacterial cell or the host-derived response, respectively) serving several overlapping roles. For instance, master regulators (e.g. CovR/S and RofA-like proteins) modulate

broader virulence-related networks; metabolite-sensitive factors (e.g. CcpA and Mga sensing carbohydrates or Rsh and CodY sensing amino acid/nitrogen supplies) adjust responses to nutrient availability; metabolic homeostasis regulators (e.g. VicR/S, MtsR, and CiaH/R) influence expression of genes to promote colonization; environmental and host immunity-responsive regulators control responses to multiple different external cues (e.g. PerR, CiaH/R for oxidative stress stimuli; Rgg2/3, Sil involved in quorum sensing mechanisms; Ihk/Irr, CovR/S, LiaS/F/R for neutrophil escape and survival). While direct evidence linking DNA methylation and virulence in GAS remains limited, restriction-modification (RM) systems (notably Type I systems encoded by the *hsdRMS* locus) can alter gene expression via DNA methylation and phase-variable regulation in GAS and several other streptococci(Nye et al. 2019, DebRoy et al. 2021). GAS possesses an intact DNA methylation Type I RM system, whereas phase-variable systems are present in *Streptococcus pneumoniae* (DebRoy et al. 2021).

In this section, virulence determinants are classified according to their subcellular localization (and secretion signal) and intrinsic biochemical activity. Specifically, small proteins (or peptides) released into the extracellular *milieu* characterized by the presence of a signal peptide in absence of an LPXTG motif exerting effects at a distance are enlisted among secreted exotoxins (e.g. superantigens, pore-forming toxins); proteins carrying cell-wall anchoring signals, such as the LPXTG motif and active while associated with the bacterial surface or cell envelope are enlisted among surface-anchored/cell-envelope proteases; microbial enzymes with *in vitro* demonstrated catalytic activity, as well as nonenzymatic bacterial factors that activate host enzymes, are enlisted among surface-anchored/cell-envelope proteases enzymes and host-activating factors.

M protein

The M protein is encoded by the *emm* gene and represents the main virulence determinant of *S. pyogenes,* significantly conferring resistance to phagocytosis in the absence of type-specific antibodies (Lancefield 1962). It is a long fimbrial adhesin occurring as a dimer on bacterial surface (Swanson and Gotschlich) and expressed by nearly all GAS isolates. M proteins are characterized by a common basic structure that includes a signal peptide, a hypervariable N-terminal signal sequence, a modular central region, and a highly conserved C-terminus ending in a LPXTG motif (Haanes-Fritz et al. 1988, Smeesters et al. 2010) (Fig. 1). The signal peptide marks the M protein for export and allows its secretion to the division septum leading to coating of the entire cell surface, while the exposed hypervariable N-terminus promotes antigenic diversity and bacterial evasion by the host immune system (Carlsson et al. 2006, Smeesters et al. 2010). The central helical rod region of M proteins consists of repeat sequences (A, B, C, and D repeats) with variable length and organization correlating with the *emm* typing pattern (100–102). Homologous recombination within the repeats is associated with size variation among and within M proteins of the same and different serotypes, eventually contributing to immune evasion by reducing the number of potential surface epitopes (Fischetti et al. 1985, Fischetti 1989). Besides the *emm* gene (Hollingshead et al. 1986), *S. pyogenes* genome generally contains *emm*-related genes namely *mrp* and *enn* encoding M-like proteins (105–107). The *emm*-like genes are present within the chromosomal locus designated multiple gene activator (*mga*) regulon also called virulence regulator and M protein RNA yield, spanning from the *mga* gene to the end of the *scpA* gene and harboring a varying number *emm* and *emm*-like genes classified as five genetic patterns (A–E) (Frost et al. 2020). The M protein can be differentiated in two classes by reactions with specific antibodies, which bind to C repeat regions of some strains (class I) but not with that of others (class II) (Bessen et al. 1989). While adhesion to human keratinocytes and internalization into epithelial tissues may be mediated by an M protein-CD46 interaction (Oliver et al. 2008), and M proteins can also bind fibronectin facilitating the early stages of *S. pyogenes* infection (McMillan et al. 2013), there is not an universal M protein-mediated mechanism for adhesion to eukaryotic cells (Ryan and Euler 2022). The central region of M proteins binds to several fibrinogen molecules simultaneously resulting in the formation of a large complex that stimulates heparin-binding protein release, inflammation, and vascular leakage (Macheboeuf et al. 2011, Herwald et al. 1996). M proteins possess antiopsonic properties with different M protein isoforms inhibiting complement deposition on the bacterial cell surface through various mechanisms (Bisno 1979). M proteins bind to C4b-binding protein and to factor H-like-1 retaining the complement regulatory functions of these proteins and reducing the availability of binding sites for serotype-specific antibodies (Johnsson et al. 1996, 1998).

T protein

The trypsin-resistant surface T protein has been used for *S. pyogenes* typing and is encoded by the *tee* gene (Lancefield and Dole 1946, Schneewind et al. 1990). T proteins are part of the pilus structure of the bacterium, which is composed of multiple subunits that bear a C-terminal LPXTG-like motif (Mora et al. 2005) (Fig. 1). Genes responsible for pilus structure and biosynthesis are clustered in the FCT (fibronectin- and collagen-binding proteins and T antigen-encoding) locus, which is flanked by the conserved *hsp33* and *spy0136* genes and varies in composition and sequence defining nine different FCT types (Falugi et al. 2008). The FCT locus encodes transcriptional regulators (RofA, Nra, and MsmR), fibronectin-binding proteins (SfbI/F1, F2), the pilus-related proteins, namely the BP/FctA/Tee backbone protein, and two ancillary proteins (AP1/Cpa and AP2/FctB), constituting the pilus tip and base, two sortase enzymes (SrtB, SrtC1 or C2), and a signal peptidase (SipA/LepA) (Bessen and Kalia 2002, Mora et al. 2005). The SrtB and SrtC sortases cleave the membrane-attached precursor pilus proteins at their LPXTG-like sorting signals, while the covalent attachment of pilus to cell wall is mediated by sortase A, which is encoded at a different locus in the genome (121–123). *Streptococcus pyogenes* pilus plays a role in both formation of biofilm and microcolonies, and adhesion to human tonsillar epithelium, pharyngeal cells, and primary human keratinocytes (Abbot et al. 2007, Manetti et al. 2007). Different FCT types are characterized by different capacity of forming biofilms and microcolonies (Köller et al. 2010). Additionally, *S. pyogenes* pilus contributes to immune evasion (Tsai et al. 2017: 200, Chen et al. 2020).

Streptococcal pyrogenic exotoxins

Superantigens are potent mitogenic exotoxins secreted by *S. pyogenes* and a few other bacterial species, capable of simultaneously binding to major histocompatibility complex (MHC) class II on antigen presenting cells and the T-cell receptor of T cells (Fraser and Proft 2008). Streptococcal superantigens are proteins that range from 22 to 29 kDa in size, highly resistant to proteases and heat denaturation, which contain signal peptides that are cleaved after extracellular secretion (Baker and Acharya 2004). Currently, at least 14 *S. pyogenes* superantigens have been characterized, most of which are encoded by lysogenic bacteriophages. Typically, *S. pyogenes* strains contain between three and six distinct superantigens (Shannon et al. 2019). The first superantigens

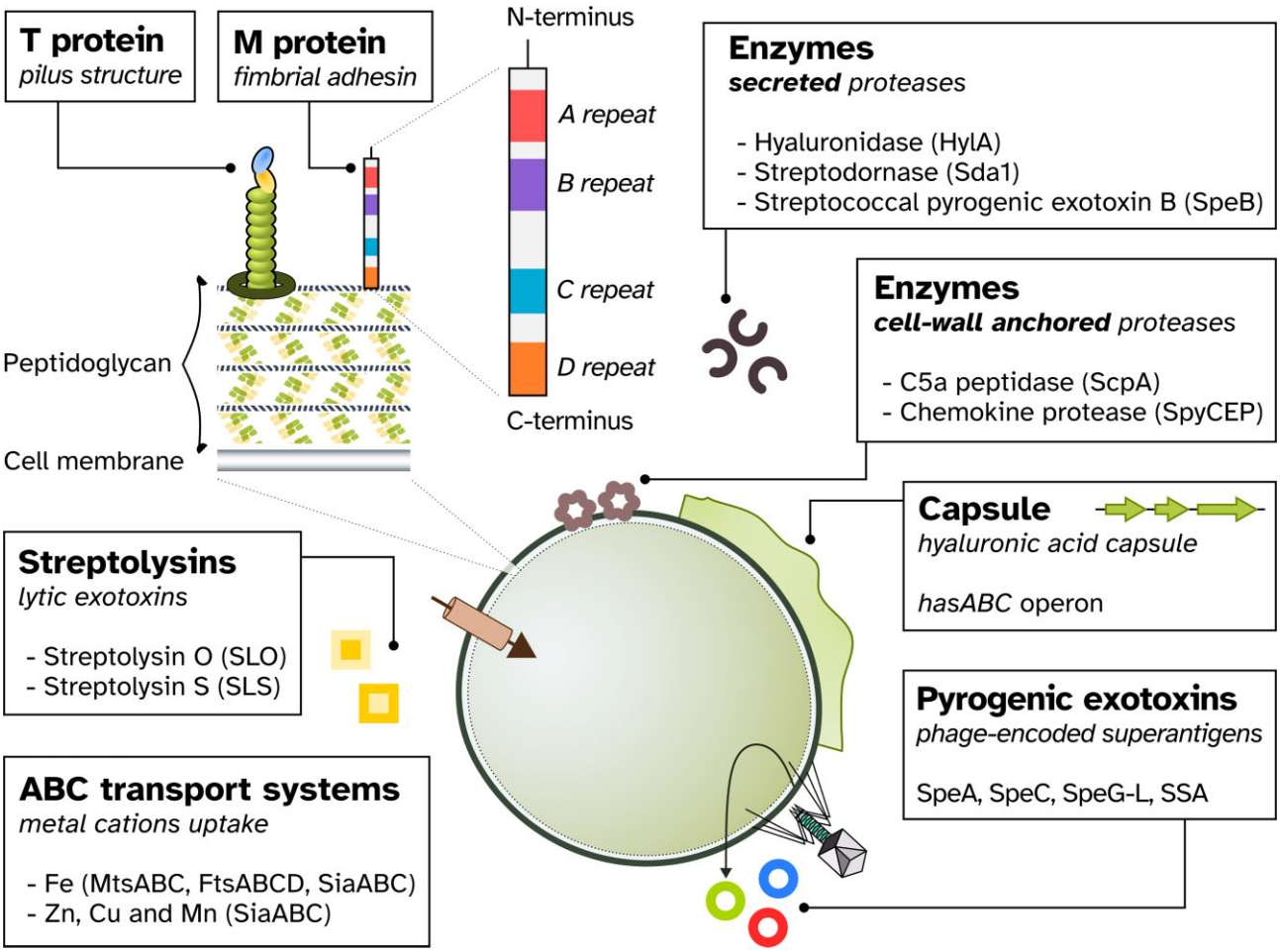

Figure 1. Overview of major virulence factors in *S. pyogenes*. Surface-associated structures include M- (fimbrial adhesin) and T-protein (trypsin-resistant pilus structure), both historically used for typing *S. pyogenes*. The hyaluronic acid capsule, encoded by the *hasABC* operon, contributes to immune evasion. Among enzymes, secreted proteases include hyaluronidase (HylA, in most isolates carried as nonfunctional variant), streptodornase (Sda1), and SpeB, whereas cell-wall anchored proteases include the C5a peptidase (ScpA), and chemokine protease (SpyCEP). Lytic exotoxins, streptolysin O (SLO) and streptolysin S (SLS), promote host cell lysis. ABC transporters mediate metal uptake, mainly iron (Fe) but also zinc, copper, and manganese (Zn, Cu, and Mn, respectively). Pyrogenic exotoxins (SpeA and SpeC) act as superantigens.

identified in *S. pyogenes* were the streptococcal pyrogenic exotoxins (SPE) A, B, and C. However, while all three share the ability to induce fever (pyrogenicity) when injected into rabbits and to enhance susceptibility to endotoxic shock (Kim and Watson 1970: 196, Dick and Dick 1983), only the first (i.e. *speA*) and the latter (i.e. *speC*) are pyrogenic exotoxins *sensu stricto* while SpeB is a chromosomally encoded protease and will be discussed in the "Enzymes" paragraph. SpeA (~25 kDa) and SpeC (~24.3 kDa) are encoded by phages and are produced by *S. pyogenes* strains isolated from patients with STSS (Bohach et al. 1990, Musser et al. 1991). Four alleles of *speA* have been described, of which *speA3* codes for a more mitogenic toxin with higher affinity for MHC class II binding than other alleles (Kline and Collins 1996, Papageorgiou 1999). SpeC is a dimer molecule that binds only to the beta chain of MHC class II by a zinc-mediated mechanism similar to staphylococcal enterotoxin A (Li et al. 1997).

Streptolysins (O and S)

The secreted virulence factors of *S. pyogenes* include streptolysin O (SLO) and streptolysin S (SLS) displaying hemolytic activity on erythrocytes and numerous other activities on other cell types (Barnett et al. 2015). SLO is an oxygen-labile pore-forming exotoxin, which is encoded by the conserved *slo* gene and secreted by nearly all GAS isolates during exponential and early stationary phase (Kehoe 1987, Barnett et al. 2015). SLO is a cholesterol-dependent cytolysin, which disrupts the cytoplasmic membrane of a wide range of cell types including erythrocytes, leukocytes, macrophages, platelets, epithelial cells, and various tissue culture cell lines (150–152). Additionally, cytotoxic effect is enhanced by NAD-glycohydrolase (NADase), an enzyme cotranscribed with SLO and actively translocated by SLO itself into the cytosol of human epithelial cells to deplete energy reserves and promote host cell injury (Michos et al. 2006, Madden et al. 2001). SLO also acts synergistically with the *S. pyogenes* scarlet fever-associated superantigen SSA by inducing the release of intracellular glutathione from host cells, which promotes the conversion of SSA into the active monomer form (Brouwer et al. 2020). Furthermore, SLO has several important roles in intracellular trafficking and survival of *S. pyogenes*: (i) blocks clathrin-dependent internalization into keratinocytes, (ii) promotes cell internalization via a pathway that involves light chain 3 (LC3)-associated phagocytosis (Cheng et al. 2019), (iii) allows early endocytic compartment escape and access to cytosol (O'Neill et al. 2016), (iv) acts synergistically with NADase by inhibiting intracellular trafficking of *S. pyogenes* to lyso-

somes, and (v) promotes Golgi fragmentation thus disrupting cell–cell junctions and increasing permeability of epithelial barrier (Bastiat-Sempe et al. 2014, Nozawa et al. 2021).

SLS is an oxygen-stable pore-forming exotoxin secreted by nearly all GAS at stationary phase, which is mainly responsible for the beta-haemolysis surrounding *S. pyogenes* colonies cultured on blood agar plates (Yoshino et al. 2010). SLS is related to class I bacteriocin family and is produced by a nine-gene genetic locus (*sagABCDEFGHI*) with *sagA* encoding for the SLS precursor and the downstream genes encoding proteins involved in posttranslational modifications, heterocycle formation, processing, and export of the mature SLS exotoxin (Nizet et al. 2000, Molloy et al. 2011). Unlike SLO, SLS does not require membrane cholesterol for activity; it is related to class I bacteriocin-type peptides and exerts broad cytolytic and signaling effects via a distinct, small-peptide mechanism. The mature SLS displays a broad-spectrum cytolytic toxin that induces irreversible osmotic lysis of erythrocytes, lymphocytes, neutrophils, platelets, subcellular organelles (e.g. lysosomes and mitochondria), and several other mammalian cell types (Betschel et al. 1998). In addition, SLS may influence host cell signaling pathways at sublytic concentration during infections as it induces macrophage cell death through increased glycogen synthase kinase-3beta activity resulting in mitochondrial damage (Tsao et al. 2019), while in epithelial keratinocytes SLS inhibits Akt signaling activating the p38 MAPK cascade, NF-kB proinflammatory signaling, and caspase-independent programmed cell necrosis (Flaherty et al. 2015). Finally, SLS promotes *S. pyogenes* translocation across the epithelial barrier via degradation of epithelial intercellular junctions in concert with the host cysteine protease calpain (Sumitomo et al. 2011).

Enzymes

Streptococcus pyogenes produces and secretes several proteases that directly impact pathogenesis, mainly by targeting molecules of the host immune system, allowing the bacteria to evade the immune response or exploit it to its own advantage (Potempa and Pike 2009).

Hyaluronidase

Secreted virulence factors of *S. pyogenes* include the chromosomally encoded hyaluronate lyase HylA and two or more bacteriophage-encoded hyaluronidase (HylP, HylP2, and so on) (Hynes et al. 2000, El-Safory et al. 2011). The *hylA* gene is present in all *S. pyogenes* strains, but most of the HylA proteins are enzymatically inactive due to a point mutation that causes a substitution of aspartic acid with valine (Hynes et al. 2009) and a lesser fraction of GAS displays a third HylA variant characterized by a D199G substitution whose enzymatic activity has not been assessed so far.

Active HylA likely allows the bacterium to utilize host hyaluronic acid or its own hyaluronic capsule as a potential energy source (Rivera Starr and Engleberg 2006). Bacteriophage-associated hyaluronidases are not homologous to HylA and contain a variable region of collagen-like Gly-X-Y repeating units, which likely contribute to the enzyme structure stability (Hynes et al. 1995). HylP and HylP2 likely allow phage penetration of *S. pyogenes* hyaluronic capsule and attachment to phage receptors (Baker et al. 2002).

Streptodornase

The bacteriophage encoded Sda1, also known as streptodornase is a potent *S. pyogenes* DNase, which contributed to resurgence of *S. pyogenes* severe infections in the late 1980s and early 1990s by the M1T1 clone (Sumby et al. 2005a: 20). The expression of *sda1* is negatively regulated by CovR/S in standard conditions, while under oxidative stress it is regulated by the peroxide-induced regulator PerR (Wang et al. 2013). Sda1 degrades the chromatin component of DNA-based neutrophil extracellular traps facilitating bacterial evasion of neutrophil capture (Buchanan et al. 2006) and prevents the TLR9-mediated recognition of GAS by host innate immune cells (Uchiyama et al. 2012). Interestingly, it was demonstrated that the acquisition of the *sda1*-carrying bacteriophage by the laboratory strain SF370 was not sufficient to induce a hypervirulent phenotype, suggesting that the M1T1 clone virulence derives from the combination of several unique chromosomal mutations and HGT events (Venturini et al. 2013).

SpeB

Despite its "superantigen" appellative, which was given on account of scarlet fever clinical manifestations (Watson 1960), SpeB is a chromosomally encoded, broad-spectrum cysteine protease. SpeB is secreted as a ∼40 kDa zymogen that is autoprocessed to an active ∼28 kDa protease (Doran et al. 1999). It is chromosomally encoded and, while it can be identified in most *S. pyogenes* genomes, its degree of expression varies from strain to strain depending on the type of infection (Yu and Ferretti 1991, Ly et al. 2017).

SpeB cleavage activity affects a wide range of proteins, leading to immunomodulating effects active against several proteins including interleukin-1beta, immunoglobulins, fibrinogen, fibronectin, kininogens, and metalloproteases (111, 143–145). Additionally, SpeB is involved in the proteolytic destruction of complement factors and chemokines, thereby modulating innate immune responses and tissue damage. (Egesten et al. 2009, Honda-Ogawa et al. 2013).

Host activators and cell-wall anchored proteases

Streptokinase

Streptokinase (Ska) is a single-chain 414-amino acid-secreted protein that acts as a plasminogen activator rather than a classical bacterial hydrolase by converting the host single-chain glycoprotein zymogen (plasminogen) to the proteolytically active plasmin (Bajaj and Castellino 1977). Although Ska itself does not possess an intrinsic proteolytic active site, it forms a complex with host plasminogen that induces the conformational activation of plasminogen to plasmin, thereby promoting plasmin-mediated proteolysis of extracellular matrix and host defense proteins. Streptokinase binds to plasminogen and induces a conformational change in the latent active site resulting in the production of an enzymatically active streptokinase-plasminogen complex, which proteolytically converts its substrate, plasminogen, into plasmin (Boxrud et al. 2000). Streptokinase contains three distinct domains (α, β, and γ) of which the β-domain exhibits significant sequence variability among *S. pyogenes* strains resulting in the use of distinct plasminogen activation pathways that impacts the pathogenic potential (171–173). Plasminogen activation by streptokinase leads to the plasmin-mediated degradation of the extracellular matrix, tissue barriers, and fibrin networks, facilitating bacterial dissemination throughout the human body (Walker et al. 2005). Additionally, streptokinase mediates plasmin degradation of host innate immune effectors promoting bacterial evasion of host immune system and triggers the release of bradykinin which contributes to inflammation during *S. pyogenes* infection (Nitzsche et al. 2015, 2016).

C5a peptidase

Streptococcal C5a peptidase (ScpA) is a serine protease that specifically cleaves the human C5a complement factor inhibiting its serum chemotactic activity (188–190). More recently, it was demonstrated that ScpA has proteolytic activity against both C3 and C3a complement factors, generating nonfunctional peptides (Lynskey et al. 2017). ScpA contains a catalytic triad motif (Asp130, His193, and Ser512) that is critical for its enzymatic activity and an LPXTG motif at the C-terminus, which permits the anchoring to the bacterial cell wall (O'Connor and Cleary 1986, Stafslien and Cleary 2000). ScpA is processed from a precursor to mature peptide through autocatalytic cleavage of 31 N-terminal amino acids, resulting in the formation of a catalytically active protein (Cleary et al. 1992). ScpA cleaves C5a at the His67 residue, releasing the C-terminus which makes the protein inactive, impairing the activation and recruitment of neutrophils to the site of infection and consequently favoring bacterial persistence and dissemination (Cleary et al. 1992). ScpA is also an invasin, which mediates the attachment of *S. pyogenes* to endothelial and epithelial cells and further contributes to virulence independently from its complement proteolytic activity (Cheng et al. 2002, Lynskey et al. 2017).

Chemokine protease

The *S. pyogenes* cell envelope protease (SpyCEP also known as ScpC, PrtS) is a cell wall-associated serine protease with immunemodulatory effects and represents one of the main virulence factors contributing to pathogenesis in iGAS disease (Honda-Ogawa et al. 2013). SpyCEP cleaves the C-terminal α-helix of CXC human chemokines (CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8), both at the site of infection and systemically reducing CXCR1 and CXCR2-mediated neutrophil chemotaxis (Honda-Ogawa et al. 2013). Additionally, SpyCEP cleaves the human antimicrobial peptide LL-37 reducing its specific neutrophil chemotaxis (Biswas et al. 2021). SpyCEP is a member of the S8 family of subtilisin-like protease containing a catalytic triad (aspartate, histidine, and serine residue), which is autocatalytically cleaved during maturation into two distinct polypeptides (a 30 kDa N-terminal and a 150 kDa C-terminal), which are then noncovalently reassociated to reconstitute the active enzyme (Zingaretti et al. 2010, McKenna et al. 2020).

Capsule

Most clinical GAS isolates produce a hyaluronic acid capsule, resulting in a mucoid colony morphology when cultured on solid media. Hyaluronic acid capsule is a major virulence factor of *S. pyogenes*, which confers resistance to phagocytosis and mediates the attachment to pharyngeal and epidermal keratinocytes via CD44 binding (197–199). The capsule consists of hyaluronic acid, a high molecular mass linear polymer composed of repeating units of *N*-acetylglucosamine and glucuronic acid, which is structurally identical to the hyaluronic acid found in many higher animals (Kendall et al. 1937). Capsule production is associated with the 4.2 kb chromosomal *has* operon, which contains the *hasA*, *hasB*, and *hasC* genes, each encoding an enzyme involved in hyaluronic acid synthesis (Dougherty and van de Rijn 1992). Additionally, capsule biosynthesis is negatively regulated by the CsrR regulator of the two-component system CsrRS (also known as CovRS) in a serotype- and strain-specific manner (Levin and Wessels 1998, Sugareva et al. 2010). The *has* operon is highly conserved among *S. pyogenes* strains but absent in M-type 4 and 22 isolates, which conversely possess a functional hyaluronidase lyase enzyme that is rendered nonfunctional in other *S. pyogenes* through a point mutation (Hynes et al. 2009, Flores et al. 2012, Henningham et al. 2014). The *has* operon was deleted also in a new clade of *emm89* GAS emerged in UK in 2008 characterized by high-level of expression of streptolysin O and NAD-glycohydrolase (Turner et al. 2015a, Zhu et al. 2015). M-type 28 and 87 isolates contain a *has* operon, but do not produce capsule due to a nonsense point mutation in the *hasA* gene (Flores et al. 2019). Some *S. pyogenes* strains contain the insertion sequence IS1239 upstream of the −35 site of the *hasABC* operon, however, the presence of the insertion sequence is not likely to influence the expression of the capsule (Ashbaugh et al. 1998).

Biofilm formation

Biofilm is a mono- or polymicrobial community embedded within an extracellular polymers matrix, which is firmly attached to a substratum. Microbes develop biofilms to endure unfavorable environmental conditions such as, for *S. pyogenes*, acidification.

Historically, the ability of *S. pyogenes* to form biofilm has been reported in impetigo skin lesions and human tonsils carriage without a clear understanding of its development and contribution to clinically relevant manifestations (Akiyama et al. 2003, Roberts et al. 2012).

One of the main causes leading to this lack of knowledge is the reliance on *in vitro* static models, which overlook the complex interactions between the host, the pathogen, and the environmental context: classic plate-based assays highlight the role of some virulence factors (e.g. the M protein, capsule, pili, and SpeB) in biofilm formation, but they reflect microbial behavior on abiotic surfaces (Vyas et al. 2019), a limitation that results in discrepancies between what can be observed *in vitro* and what happens *in vivo* (Skutlaberg et al. 2022).

Yet, starting from clinical reports, the role of biofilm formation in necrotizing soft tissue infections, such as necrotizing fasciitis is coming to light (Siemens et al. 2016).

Above all virulence factors, the M protein has been object of in-depth analyses. M protein deficient *S. pyogenes* isolates cannot form biofilm in any condition (stable or flow), even after prolonged time intervals and the inclusion of trypsin, which cleaves surface proteins without affecting *S. pyogenes* growth, has the same inhibitory effect (Cho and Caparon 2005, Courtney et al. 2009). A plausible explanation is the interaction between the M protein and lipoteichoic acid, which contributes to biofilm hydrophobicity (Courtney et al. 2009).

While displaying a high variability among strains, biofilm growth and structure correlate with the *emm* type as well: M41 and M28 isolates produce a thick biofilm characterized by low cellular density, with the latter displaying marked interisolate inconsistencies (Skutlaberg et al. 2022); M1 isolates form a thin and smooth biofilm characterized by a single, high cell density, layer with abundant bacterial-associated extracellular matrix with a remarkably low interstrain variability (Skutlaberg et al. 2022); M3 isolates, in contrast with other *emm* types, require collagen to produce biofilm and are not able to develop it on abiotic surfaces (Wojnowska et al. 2025). Moreover, the impact of *emm* gene inactivation on biofilm varies according to the M type: M2 and M4 strains are mildly or not affected, while in M18 and M49 strains biofilm production decreases by 50% (Courtney et al. 2009).

Multiple pili-associated components, such as sortase SrtA, T protein, and Ancillary Protein 1 are involved in biofilm formation, promoting *S. pyogenes* switch from planktonic to biofilm growth and thickness (Manetti et al. 2007, Nakata et al. 2009). Capsular effects on *S. pyogenes* biofilm formation appears to be strain specific, although both an impairment in capsule production due to

deletion of the hyaluronate synthase *hasA* gene and an overproduction of the capsule resulted in the inhibition of biofilm formation (Heath et al. 1999, Cho and Caparon 2005, Sugareva et al. 2010). The AspA (A *Streptococcus* surface protein A) cell surface-anchored protein contributes to adherence and biofilm formation of *S. pyogenes* in the presence of a salivary glycoprotein substratum (Maddocks et al. 2011). The extracellular protein Streptococcal collagen-like protein 1 (Scl1) is involved in *S. pyogenes* adherence and virulence, also by increasing biofilm formation (Lukomski et al. 2000). The Rgg-SHP quorum-sensing pathway consisting of the Rgg (regulator gene of glucosyltransferase)-family of cytoplasmic receptors for intercellular signaling peptides is highly conserved in *S. pyogenes* and is involved in biofilm formation (Chang et al. 2011b). Environmental factors, such as anaerobiosis and glucose availability positively affect the formation of biofilm (Baldassarri et al. 2006, Thenmozhi et al. 2011).

Iron acquisition

Iron acquisition is mediated by three ABC transporters, namely the metal transporter of *Streptococcus* (MtsABC), the Shr/Shp/SiaABC system, and FtsABCD transporter that utilize energy from Adenosine triphosphate (ATP) hydrolysis to actively transport free iron or haemoproteins across the cell membrane. The MtsABC transporter has a broad specificity for metal cations and is involved in the uptake of zinc, copper, and manganese in addition to iron (Janulczyk et al. 1999, 2003). This ability is conferred by the multiple ligand specificity of its accessory lipoprotein MtsA (Janulczyk et al. 1999, 2003). The Shr/Shp/HtsABC system consists of two surface proteins Shr and Shp and the ABC transporter SiaABC (also known as Hts), which mediates the uptake of heme iron (223–225). Heme iron is released from met-haemoglobin by Shr and placed on the lipoprotein SiaA by Shp, then it is translocated into the cytoplasm by the membrane permease SiaB in an ATP-dependent manner (Zhu et al. 2008). Recently, another three genes of the *sia* operon, *siaFGH*, have been demonstrated to constitute a novel heme importer (Chatterjee et al. 2020). The FtsABCD transporter is composed of four subunits (FtsA, FtsB, FtsC, and FtsF) and is responsible for the Fe^{3+} ferrichrome uptake in *S. pyogenes* (Smoot et al. 2001, Hanks et al. 2005).

Antimicrobial resistance

Chromosomal mutations

Mutation is the main mechanism of genetic variability and evolution, since it does not require an organism to establish any kind of productive interaction with foreign genetic elements. In bacteriology, albeit less common than the acquisition of mobile resistance determinants, point mutations contribute to antimicrobial resistance through alteration in drug-binding sites or enzyme structures (Baquero et al. 2009).

Penicillin binding protein 2x variants

Streptococcus pyogenes has remained universally susceptible to beta-lactams since the beginning of the antibiotic era, yet a growing body of evidence indicates that strains with elevated minimum inhibitory concentration (MIC) are emerging. These remain below clinical resistance breakpoints but suggest early-stage adaptation deserving monitoring. A comprehensive surveillance study of 13 727 invasive GAS isolates in the USA from 2015 to 2021 identified PBP2x variants in 2.5% of strains, many exhibiting MICs 4–8 times higher than the wild-type baseline (Chochua et al. 2022). Key PBP2x substitutions include A397V, T553K, M593T, and P601L, all positioned in the transpeptidase domain within or in close proximity of the beta-lactams binding pocket (Figure S1). The T553K substitution, identified in an *emm*43.4 background, results in elevated ampicillin and amoxicillin MICs (0.25–0.5 µg/ml), values that approach epidemiological cutoff thresholds while remaining below resistance breakpoints (Chochua et al. 2022). M593T and P601 L are particularly widespread, found across several *emm* types including *emm*4, *emm*1, and *emm*75, and are associated with reduced susceptibility to multiple beta-lactams. Other reports from Japan, Iceland, Guyana, and Ethiopia confirm similar amino acid substitutions in either PBP2x or PBP1a, suggesting convergent evolution in response to beta-lactam use (232–234). A global genomic analysis revealed that while most isolates still possess highly conserved PBPs, the occurrence of these mutations, although infrequent, warrants attention (Hayes et al. 2020). Mechanistically, these changes reduce the affinity of beta-lactams for PBP2x active site, enabling partial resistance while preserving bacterial fitness. Interestingly, none of the PBP2x variants identified to date possess acquired beta-lactamase genes, and resistance appears to follow a stepwise accumulation of point mutations, analogous to mechanisms described in *S. pneumoniae* (Yu et al. 2023).

Despite low prevalence and retained clinical susceptibility, these findings underscore the importance of continued genomic surveillance. Indeed, the presence of lower susceptibility-associated PBP2x mutations, especially in high-risk clones with cooccurring macrolide resistance, could complicate therapy in patients allergic to penicillin and may eventually erode the efficacy of first-line treatments if left unmonitored.

Chromosomal mutations associated with resistance to other antimicrobial agents

GAS isolates have been considered intrinsically resistant to folate pathway inhibitors (specifically to the commonly used combination of trimethoprim–sulfamethoxazole, also called cotrimoxazole, SXT) for a long time, but this understanding was a consequence of primordial AST approaches (Eliopoulos and Wennersten 1997). In fact, under standardized conditions, most GAS isolates are susceptible to SXT (Bowen et al. 2012). Changes in the gene encoding dihydropteroate synthase *folP* are a common sulfamethoxazole resistance mechanism. Specifically, some resistant isolates display a 2.3-kb chromosomal fragment encoding a dihydropteroate synthase variant with 30 amino acid substitutions compared to the wild-type and characterized by a lower affinity for sulfamethoxazole (Swedberg et al. 1998). The divergence of high- and low-affinity dihydropteroate synthase variants hints to the occurrence of a recombination event rather than stepwise point mutations alone. The SXT combination also contains trimethoprim, which inhibits a later step of the folate pathway by binding to the dihydrofolate reductase enzyme. However, the I100 L substitution in the dihydrofolate reductase protein leading to trimethoprim resistance has been reported (Bergmann et al. 2012, 2014).

The main drivers of fluoroquinolone resistance in GAS are point mutations in genes encoding type II topoisomerases, *parC* and *gyrA*. Relevant mutations occur in small fragments of the genes, which are called quinolone resistance-determining regions (QRDRs) and two different, yet nonmutually exclusive, evolutionary pathways have been proposed to describe the development of fluoroquinolones resistance in GAS. On one hand, there are observations of a consistent pattern in fluoroquinolone resistance development, characterized by initial mutations in ParC (typically in the serine in position 79) followed by additional GyrA changes (typically in the serine in position 81 and in the methionine in

position 99); interestingly, *parC* mutations confer low-level resistance, but highly resistant strains possess mutations also in *gyrA* (Alonso et al. 2007, Wajima et al. 2013). On the other hand, it has been proposed that low affinity *parC* alleles can be acquired via interspecies recombination with *S. dysgalactiae* subsp. *equisimilis*, plausibly following bacteriophage-mediated transduction (Pletz et al. 2006). However, the number of GAS isolates resistant to fluoroquinolone is rather small, and robust theories on these trajectories are hence difficult to infer.

Macrolide–lincosamide–streptogramin B phenotypes, instead, are most often a consequence of acquired methylases (*erm*) or efflux pumps (*mef*). Substitutions and/or small deletions in ribosomal proteins contributing to structure and function of the peptide exit tunnel have been associated with intermediate macrolide resistance in other *Streptococcus* species, but not in GAS (Wilson 2014), and high-level resistance from point mutations over multiple *rrn* operons in the V domain of the 23S rRNA is rare (Jalava et al. 2004, Richter et al. 2005).

In GAS, rifampin resistance is rare and can be observed in a small fraction of isolates. However, mutations in an 81-bp conserved fragment of the *rpoB* gene encoding the RNA polymerase beta subunit (defined as rifampin-resistance-determining region, RRDR) can lead to high-level rifampin resistance (Aubry-Damon et al. 2002, Herrera-Leon et al. 2002).

Acquired antimicrobial resistance genes

The most common class toward which GAS isolates acquire resistance are tetracyclines, with *tet*(M) often associated with macrolide resistance (Hammerum et al. 2004, Nielsen et al. 2004). The high prevalence of tetracycline resistance determinants reflects the global overuse of antibiotics belonging to this class (e.g. as prophylactic agents or growth promoters in animal feeds) (Sanderson et al. 2005, Wu et al. 2024), which fuels the spread of the MGEs carrying these genes (Lu et al. 2017).

Macrolide resistance is generally associated with the *erm*(A) and *erm*(B) genes, often identified in isolates with *tet*(M) hinting to multiple independent acquisition events (Gherardi et al. 2015).

Consistently with the low clinical use of aminoglycosides against members of the genus *Streptococcus*, genes encoding aminoglycoside modifying enzymes such as the *aph(3′)-III—ant(6)-Ia* pair are uncommon, but notable for the genomic plasticity and the potential to expand under selective pressure (Southon et al. 2020).

Typing methods

Serotyping

Historically, three antibody-dependent serotyping schemes have been used to characterize *S. pyogenes*, namely M-typing, T-typing, and serum opacity factor (SOF)-typing, all based upon the antigenic specificity of surface proteins covalently linked to cell wall, which are both key virulence factors and targets of host protective immunity (Griffith 1934, Lancefield and Dole 1946, Johnson and Kaplan 1993).

The Lancefield M-typing system is dependent on the preparation of type-specific antisera against the M proteins, which are the major antiphagocytic virulence factors of *S. pyogenes* (Lancefield and Dole 1946). Different antigenic specificities arise from N-terminal sequence variations in the M proteins, which are detected by precipitation typing (Beachey et al. 1981, Fischetti 1989). M-typing may not be possible for certain *S. pyogenes* isolates due to the absence of M protein expression, lack of reactivity with available antisera, or the expression of a previously uncharacterized M protein (Tanna et al. 2006).

T-protein serotyping and SOF-typing are useful adjuncts to M-protein serotyping (Johnson et al. 2006). T-typing is based on the trypsin-resistant T protein, which is part of the pilus structure (Mora et al. 2005: 20) and can be performed using commercially available assays with ∼20 recognized anti-T sera. T types correlate with M types, but are less specific (Johnson and Kaplan 1993). SOF-typing is based on a fibronectin binding protein with enzymatic activity promoting serum opacification, which can be neutralized by hyperimmune antiserum (Maxted et al. 1973). Although not all *S. pyogenes* strains produce SOF, SOF-typing usually correlates well with M type, even if multiple *sof*-gene types can occur within a single M type, and the same *sof* sequence can be present in different M types (Johnson et al. 2006). Serotyping methods have been largely substituted by molecular methods based on sequencing of gene fragments (*emm* typing, MLST) or on data from whole genome sequencing.

PCR *emm* typing

At the end of the 20th century, with the increased availability of Sanger sequencing, a molecular typing system for *S. pyogenes* was developed based on PCR amplification and subsequent nucleotide sequencing of the variable 5′ end of the M protein (*emm*) gene, which is the determinant for M-typing serotype specificity (Beall et al. 1996). This system, called *emm* typing, uses conserved primers designed on the *emm* gene, to amplify a fragment of about 1 kb in length (Whatmore et al. 1994, Beall et al. 1996, Frost et al. 2020). Molecular serotyping through *emm* sequencing allows an assignment to a validated M protein gene sequence and easy identification of new *emm*-sequence types (ST) and subtypes (Facklam et al. 2002, McMillan et al. 2013). Genotype definition is based on a cutoff of <92% nucleotide identity to any other *emm* type within the 90-nucleotide sequence at *emm* 5' end, while subtypes are assigned based on single nucleotide polymorphisms (SNPs) and/or small indels within the 180-nucleotide sequence of the *emm* 5' end. Currently, the worldwide database of *emm* type specific sequences is hosted and curated by the US Centers for Disease Control and Prevention (CDC) and contains more than 275 different *emm* types identified (https://www.cdc.gov/strep-lab/php/group-a-strep/emm-typing.html).

Multilocus sequence typing

A multilocus sequence typing (MLST) scheme was developed in 2001 for molecular typing of *S. pyogenes*, demonstrating a stable association between *emm* type and MLST (Enright et al. 2000). In MLST, internal fragments of seven housekeeping genes (*gki*, *gtr*, *murI*, *mutS*, *recP*, *xpt*, and *yqiL*) are amplified by PCR, sequenced and used to evaluate genetic relationship of *S. pyogenes* strains resulting in an allelic profile referred as "sequence type" (ST). Data on allelic profiles for *S. pyogenes* strains are maintained at a user-interactive website (https://pubmlst.org/organisms/Streptococcus-pyogenes) (Jolley et al. 2018). MLST is not sufficient to discriminate different isolates within the same lineage that may be responsible for an outbreak (Turner et al. 2017).

wgMLST/cgMLST

Whole genome sequencing analysis allows the identification of emerging intra-*emm* clones with increased fitness or virulence traits, that would be otherwise undistinguishable (Lynskey et al. 2011, Turner et al. 2017). Most genome-wide analyses of *S. pyo-*

genes rely on the comparison of SNPs among isolates, which require the selection of a reference genome for read alignment and the removal of regions of recombination (Beres et al. 2017, Turner et al. 2017, Davies et al. 2019). These limitations are overcome using gene-by-gene approaches like whole-genome MLST (wgMLST) and core-genome MLST (cgMLST), which rely on a fixed set of target genes (i.e. core genes) allocated throughout the genome. Recently, a wgMLST scalable scheme for *S. pyogenes* consisting of 3044 loci was proposed showing a performance comparable to that of SNPs-based methods in distinguishing recently emerged intra-*emm*-type lineages, as well as in identifying clusters of epidemiologically and genetically related isolates associated with local, short-term outbreaks. In this scheme, the number of analysed loci can be increased when analysing closely related isolates. A different cgMLST scheme was developed using data from 1095 core alleles and proposing a threshold ≤5 allelic differences to identify *S. pyogenes* isolates that are likely to belong to the same outbreak (Toorop et al. 2023).

The role of next-generation sequencing (NGS) in *S. pyogenes* epidemiology

With the advent of high-throughput sequencing technologies, the understanding of GAS population structure, transmission dynamics, and pathogenesis has deepened significantly. The mass production of bacterial WGS data, fueled by an ever-growing implementation of NGS technologies, is revolutionizing the landscape of clinical microbiology, from prevention to surveillance. WGS is characterized by an intrinsic high-resolution power, which gets continuously integrated, and as sequencing data is more produced and disseminated, improved computational facilities and sharper bioinformatic tools are developed. The combination of these elements enables clinical microbiology laboratories to benefit from genomic information, an advantage achieved not only through direct sequencing, but also indirectly by relying on national and international surveillance networks.

WGS is rapidly transitioning from research laboratories into routine clinical microbiology workflows, revolutionizing species identification, antimicrobial-susceptibility testing, and outbreak investigation (Didelot et al. 2012): comparing SNPs across the whole genomes, WGS enables high-resolution phylogenetic analyses that distinguish outbreak clusters from sporadic cases (Tagini and Greub 2017), guiding infection-control interventions in real time or allowing for *in silico* profiling of resistance genes, leading to antimicrobial susceptibility traits prediction directly from sequence data with high concordance to phenotype-based laboratory antimicrobial-susceptibility testing (Leopold et al. 2014). Benchtop platforms can now deliver assembled bacterial genomes and perform basic clinical microbiology-oriented bioinformatic pipelines within 12 h of culture isolation (Rebelo et al. 2025), significantly reducing the time to actionable results compared to conventional methods and thus accelerating therapeutic decision-making.

Integration of WGS into diagnostic pipelines also allows for more comprehensive One Health surveillance: large-scale genomic databases aggregate thousands of isolates to monitor the emergence and global spread of high-risk clones (Fricke and Rasko 2014). In the specific case of GAS, WGS contributed to highlight the role of an emerging lineage, named $M1_{UK}$, which led the way of the postpandemic upsurge in iGAS infections (Li et al. 2023b, Vieira et al. 2024, Iden et al. 2024).

These improvements are making real-time surveillance an accomplishable task: rapid linkage of clinical isolates during hospital outbreak, WGS-inferred targeted therapy, and infection control strategies are reality. Most of these results are achieved thanks to robust and user-friendly bioinformatic pipelines and to an increasing number of accessible centralized databases. Indeed, genomic datasets are now accessible repositories for microbiologists, enabling integration between epidemiological networks and clinical laboratories.

One of the main limitations of the genome-based approaches, including the one implemented in this review is that publicly available genomes are disproportionately derived from countries with more robust sequencing infrastructures, whereas many low- and middle-income countries (LMICs) remain underrepresented due to limited research funding and institutional capacity (Struelens et al. 2024; Pronyk). This geographic bias can skew perceived lineage prevalence and diversity, particularly if endemic strains in resource-limited settings are not captured. At the same time, there is a high and intrinsic variability of WGS results, that follows from the absence of both wet and dry lab guidelines (i.e. from library-preparation protocols to quality-control and assembly pipelines) and from the absence of best-practices in metadata collection (i.e. inconsistencies in clinical or temporal information) (Quainoo et al. 2017, Fatumo et al. 2022).

Despite these caveats, we composed a dataset of 1418 selected high-quality genomes from GAS isolates spanning nearly a century (from 1932 to 2024), sampled from multiple infection sites across all inhabited continents. The comprehensive nature of this dataset provides a strong protection against bias, since the large sample size reduces the influence of any single biased subset, and cross-validation across regions and time frames enhances robustness of major evolutionary inferences. This large-scale analysis, unprecedented for GAS, was then correlated with available genome-related metadata and with the existing literature on the main epidemiological, microbiological, and clinical features of this important human pathogen.

While a plethora of bioinformatic pipelines exists for bacterial genome analysis, the bioinformatic tools employed in this review represent one of many potential approaches for future clinical microbiology implementation. These tools exemplify robust, scalable, and reproducible methods for genome quality control, population clustering, resistome profiling, and pangenome phylogeny.

To exemplify how WGS data and metadata can guide best practices in clinical and molecular microbiology, we downloaded all *S. pyogenes* genomes from the RefSeq NCBI database as of 1 May 2024, retrieving a total of 2041 nonduplicated genomes.

We then assessed completeness and contamination of this database using the "specific" neural network mode of the CheckM2 tool, (Chklovski et al. 2023) and retained only genomes with a defined collection date and isolation source for further analysis. After filtering, a total of 1418 genomes remained.

The isolation source of each sequenced strain was used as a proxy of invasiveness. Strains were classified as iGAS when recovered from normally sterile body sites or from deep/tissue specimens consistent with invasive disease, in accordance with the recommended surveillance case definitions in the Standardization of Epidemiological Surveillance of Invasive Group A Streptococcal Infections (Miller et al. 2022). Accordingly, for the present study we classified isolates obtained from blood, cerebrospinal fluid, pleural fluid, joint fluid, lower-respiratory specimens consistent with pneumonia (including pleural aspirates), and deep soft-tissue/abscess specimens as iGAS. In reference to the surveillance criteria, an exception was made. In absence of supporting clinical evidence, GAS isolates sampled from wounds were not considered as invasive, and only isolates with unequivocally defined invasive

presentations (e.g. necrotizing fasciitis) were included among soft tissue infections. This conservative approach was chosen to minimize misclassification of superficial infection as invasive disease.

Genomics of *S. pyogenes*

Population structure

While the first complete genome sequence of a strain of one *S. pyogenes* isolate dates back to 2001 (Ferretti et al. 2001), complete genome sequencing of the *S. pyogenes* type strain (NCTC 8198^T, accession number LN831034.1), has been determined much later using two independent yet equivalent approaches (i.e. PacBio-solo and Illumina + Oxford Nanopore Technologies followed by long-reads first hybrid assembly) (Salvà-Serra et al. 2020).

Here, to reconstruct the backbone of the genus, the reference genomes of all 125 recognized *Streptococcus* species were annotated and used to build a core genome alignment (Seemann 2014, Page et al. 2015), which served as base for a genus-level phylogeny (287, 287–289). *Streptococcus pyogenes* is a well-defined species within the genus, clearly distinguished from its nearest relatives in both digital DNA–DNA hybridization (dDDH) and average nucleotide identity (ANI) metrics. In particular, its closest taxa are *Streptococcus canis* (dDDH ≈ 30%, ANI ≈ 80%) and *Streptococcus dysgalactiae* (dDDH ≈ 34%, ANI ≈ 86.5%), underscoring how GAS is a taxonomically discrete species (Fig. 2, panel A).

In the last years, multiple parallelisms between GAS and *S. dysgalactiae* subsp. *equisimilis* (SDSE) have been drawn (299–302) (SDSE is represented as a red dot in Fig. 2, panel A). In spite of genomic similarities, GAS is an exclusively human pathogen while SDSE is also associated with animal infections and, as happens for other streptococcal species with wide ecological niches such as *S. agalactiae* (Crestani et al. 2024), SDSE strains identified in humans are different from those of animal origin and zoonotic transmission is an infrequent occurrence (Porcellato et al. 2021).

Subsequently, to roughly assess the structure of the *S. pyogenes* species, a representative subset of 66 genomes within the 1418 sequences database was obtained using a Mash distance approach (https://github.com/rrwick/Assembly-Dereplicator), annotated and used to build a core genome alignment (Seemann 2014, Page et al. 2015), which served as base for a general phylogeny of *S. pyogenes* (287, 287–289) (Fig. 2, panel B). Both phylogenetic figures were finalized and visually adjusted using the open-source Inkscape software.

GAS genomes display a conserved GC content of 38.4% (standard deviation, SD, 0.1%) and an average genome length of 1.8 Mb (SD 60 450 bp), encoding a mean of 1696 genes per genome (SD 75 genes) (Jespersen et al. 2024). Being a human only pathogen, GAS has a narrow ecological niche and hence lacks a relevant driver of pangenome variability (Tettelin et al. 2005); nonetheless, its pan-genome is open and dynamic, and each additional sequence increases the number of new genes and *k*-mers (Sommer et al. 2023). As a matter of fact, WGS-based studies have transformed our understanding of bacterial host range, allowing us to understand how pathogens adapt to, jump between, interact with and evolve within diverse hosts (292–294).

However, the absence of host switching in GAS still allows to perform surveillance relying on traditional source-attribution signals. To assess genomic diversity, pangenome distribution was estimated across the 1418 genomes in our dataset using PPanGGOLiN (Gautreau et al. 2020). The "exact-core" pangenome (all genes shared by every genome) is composed of 738 different genes, while the "soft-core" pangenome (genes shared by ≥95% of the genomes), consistently with transposon-sequencing analysis (Toorop et al. 2023), is composed of 1345 genes. Among the "soft-core" pangenome almost 350 genes (mostly encoding proteins involved in coenzyme metabolism, lipid transport, growth pathways, translation machinery, and cell cycle-related categories) are considered as universally essential, 300 as conditionally essential, and 750 as nonessential.

In regard to accessory genes, "shell" pangenome (here defined as those genes found in a percentage of genomes between 5% and 95%) is composed of 2205 different genes (Kislyuk et al. 2011), while the "cloud" pangenome (genes shared by ≤5% of the genomes) is composed of 8797 genes.

Pangenome variability is driven by multiple genetic dynamics, and in GAS most analyses encompassing multiple lineages support a gene-specific sweep model (i.e. alleles conferring higher fitness are transferred along with the nearby neutral sites, increasing their frequency in the population) (Qiu et al. 2022) rather than "genome-wide" clonal sweeps. This phenomenon is further confirmed by the continuous introduction of novel genetic elements into the GAS gene pool through spontaneous mutations, homologous recombination, prophage insertions, and conjugation (Takemoto et al. 2022).

Most of these exchanges involve genes improving GAS fitness in different settings, such as virulence factors and antimicrobial resistance determinants, indicating the significant role played by HGT in the evolution and adaptation of this pathogen.

Temporospatial distribution of virulence and resistance elements

GAS genomes display a marked geographic heterogeneity in terms of severity, clonal diversity, virulence gene carriage, and antimicrobial-resistance determinants (Fig. 3, panels A and B).

We scanned our database for the presence of acquired antibiotic resistance genes using RGI (Resistance Gene Identifier) based on CARD (Comprehensive Antibiotic Resistance Database, v4.0.0) (Alcock et al. 2020) and of virulence genes using the expanded virulence factor gene database (VFDB 2.0) (Dong et al. 2024) in Abricate (https://github.com/tseemann/abricate). Specific mutations associated with antimicrobial resistance (i.e. *folP* for sulfonamide resistance, QRDRs in *gyrA* and *parC* for fluoroquinolones resistance) and virulence (i.e. the *hylA* hyaluronidase gene) were assessed by a MAFFT alignment of the respective proteins.

Virulence-associated genes display an uneven global distribution, with significant differences in the median virulence factor burden which increases from Africa (~42 genes/genome) through Asia and North America (~45–47 genes/genome) to Europe and Oceania (~50–52 genes/genome) (Fig. 3, panel C). These differences are significant under an epidemiological viewpoint but do not significantly correlate with the invasiveness of GAS cases (t-test $P > .1$). However, over the past decades, the number of virulence genes carried in each GAS genome has risen significantly ($R = 0.30$, $P < .00001$), indicating ongoing acquisition of novel virulence loci (Fig. 3, panel D).

Horizontally acquired antimicrobial resistance genes also display distinct geographical distribution patterns. Asian countries report high macrolide-resistance rates (≥40%) driven by the spread of constitutive and/or inducible MLSB phenotype determinants [mainly the *erm*(A) and *erm*(B) genes, both located on Tn*916*-family MGEs] (Chancey et al. 2015, Lee et al. 2023). Europe and North America, conversely, report intermediate rates (5%–35%). Aminoglycoside-resistance genes were not detected in genomes from Africa, while they appeared in small numbers in

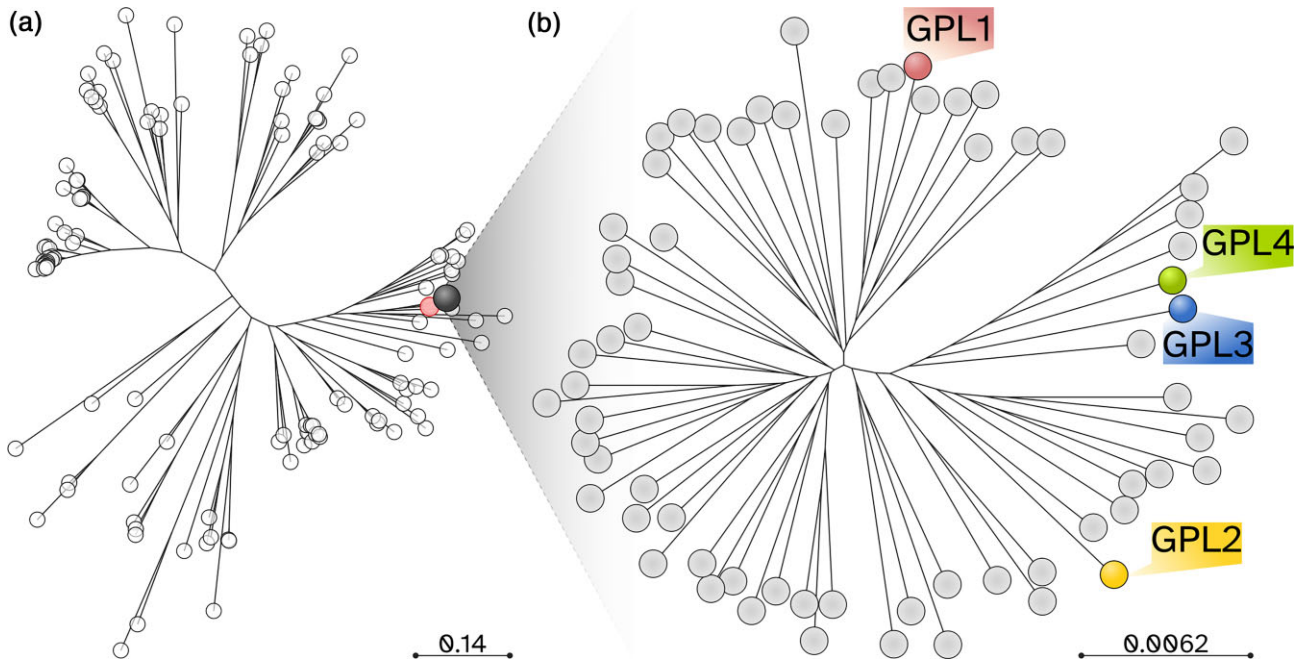

Figure 2. Phylogenetic of the genus *Streptococcus* and of *S. pyogenes*. (A) Phylogeny of 125 representative *Streptococcus* reference genomes, one per species, based on concatenated core genes. Each tip represents a different species. *Streptococcus pyogenes* is indicated by the dark gray tip, while the most similar species, *S. dysgalactiae* is represented by a slightly smaller tip. The remaining species are shown in white. Branch lengths are drawn to scale. (B) Zoomed-in view of a dereplicated subset of the 1418 *S. pyogenes* genomes. Each circle represents one genome, genomes belonging to one of the four *Global Pathogenic Lineages* (GPL) are highlighted and labelled. GPL1 (ST28/emm1); GPL2 (ST15+ST315/emm3); GPL3 (ST52/emm12); GPL4 (ST39/emm89). Branch lengths are drawn to scale.

Asia and North America (6 and 5 genomes, corresponding to 4.3% and 10%, respectively), but with a significant regional imbalance ($P < .00001$). Tetracycline-resistance genes, conversely, are highly prevalent in Africa, Asia, and North America (identified in 193/219, 64/140, and 15/47 genomes, corresponding to 88%, 45%, and 32%, respectively), while moderately present in Oceania and Europe (identified in 59/765 and 25/192 genomes, 7.8% and 13%, respectively), showing a significant regional imbalance ($P < .00001$). Acquired chloramphenicol resistance is virtually nonexistent, with only a single positive isolate identified in South America, while trimethoprim resistance genes were detected solely in a subset of genomes from Asia (6/120), indicating a highly localized reservoir.

Mutations in chromosomal genes that confer resistance phenotypes have a heterogeneous distribution as well. The alignment of the ParC and GyrA protein sequences highlighted several mutations in the QRDRs, mostly affecting serine 79 in ParC or serine 81 and the aspartic acid 99 in GyrA. Specifically, ParC codon 79 mutations are found globally, with higher prevalence in genomes from Asia (variation present in 9/140 genomes, 6.5%) and South America (variation present in 2/55 genomes, 3.7%) ($P < .0001$), GyrA codon 81 mutations occur almost exclusively in Asia (variation present in 16/140 genomes, 11.5%), while GyrA codon 99 mutations show low frequency but a wider distribution (Table 1). Alignments of the whole *parC* gene support the hypothesis that quinolone-resistant alleles may derive from interspecies recombination events with SDSE.

While these patterns suggest continent-specific selective pressures likely related to differing antibiotic practices, it is important to note that international guidelines recommend penicillin (i.e. penicillin V or amoxicillin) as the antibiotic of choice for GAS pharyngitis and only macrolides are considered as an alternative (generally only in case of documented penicillin allergy https://www.cdc.gov/group-a-strep/hcp/clinical-guidance/index.html CDC 2025a). Albeit GAS typical clinical manifestations and relatively low carriage prevalence should limit its exposure to antibiotics other than beta-lactams and macrolides (Tedijanto et al. 2018), these "cross-resistances" highlight a possible role of bystander selection during treatment of other infections, both of the respiratory tract and of other districts (Turner et al. 2015b).

These findings on temporospatial patterns of virulence and resistance elements, however, derive exclusively from publicly available WGS data, and these patterns therefore reflect the distribution of sequenced isolates rather than the true underlying prevalence in the population. This is true for studies conducted before WGS became widely adopted and still is true in regions with limited access to sequencing facilities. In both cases, reports are generally based on the detection of specific resistance and/or virulence genes using PCR (321–323). This data hence remains invisible to a purely genomic-based survey like ours, which fails to assimilate nongenomic molecular analyses data. This limitation must be considered when interpreting geographic differences, which may be driven as much by sampling intensity, sequencing policies, and by biological variation.

Evolutionary dynamics shaping global virulence patterns

Aware of the abovementioned bias, the observed lack of global homogeneity in present-day virulence features can be framed by macroevolutionary dynamics.

Founder effects

The early introduction of one or a few successful clones, the "founders," imprinted the GAS local population with their accessory gene complement. Under this scenario, the numerical dominance of the founder clone(s) produced a stable local base-

Table 1. Continental distribution of antimicrobial resistance determinants in *S. pyogenes*.

			Continent							
			Africa	Asia	Europe	North America	Oceania	South America	Total	P
Acquired resistance genes	Aminoglycoside resistance *aph(3')-III, ant(6)-Ia, ant(9)-Ia*	Absent	219	134	189	42	764	53	1401	**< .0001**
		Present	0	6	3	5	1	2	17	
	Tetracycline resistance *tet*(M), *tet*(L), *tet*(O), *tet*(T), *tet*(S), *tet*(S/M)	Absent	26	76	167	32	706	40	1047	**< .0001**
		Present	193	64	25	15	59	15	371	
	Macrolide–lincosamide–streptogramin B resistance *erm*(B), *erm*(A), *msr*(D), *erm*(T), *erm*(C)	Absent	219	95	182	34	755	50	1335	**< .0001**
		Present	0	45	10	13	10	5	83	
	Chloramphenicol resistance *catQ, catP*	Absent	219	140	192	47	765	54	1417	NA
		Present	0	0	0	0	0	1	1	
	Trimethoprim resistance *dfrG*	Absent	219	134	192	47	765	55	1412	**< .0001**
		Present	0	6	0	0	0	0	6	
Chromosomal mutations conferring resistance	FolP	WT	180	139	192	45	738	55	1349	**< .0001**
		Variant	39	1	0	2	27	0	69	
	GyrA 81	WT	219	124	192	46	765	55	1401	**< .0001**
		Variant	0	16	0	1	0	0	17	
	GyrA 99	WT	213	138	192	46	765	54	1408	**< .0001**
		Variant	6	2	0	1	0	1	10	
	ParC 79	WT	219	131	186	46	759	53	1394	**< .0001**
		Variant	0	9	6	1	6	2	24	

"WT" indicates wild-type (susceptible) alleles; "variant" indicates presence of nonsynonymous mutations associated with reduced susceptibility. The final column displays the chi-square *P*-value testing for significant differences in distribution across continents (NA = not applicable, due to extremely low counts).

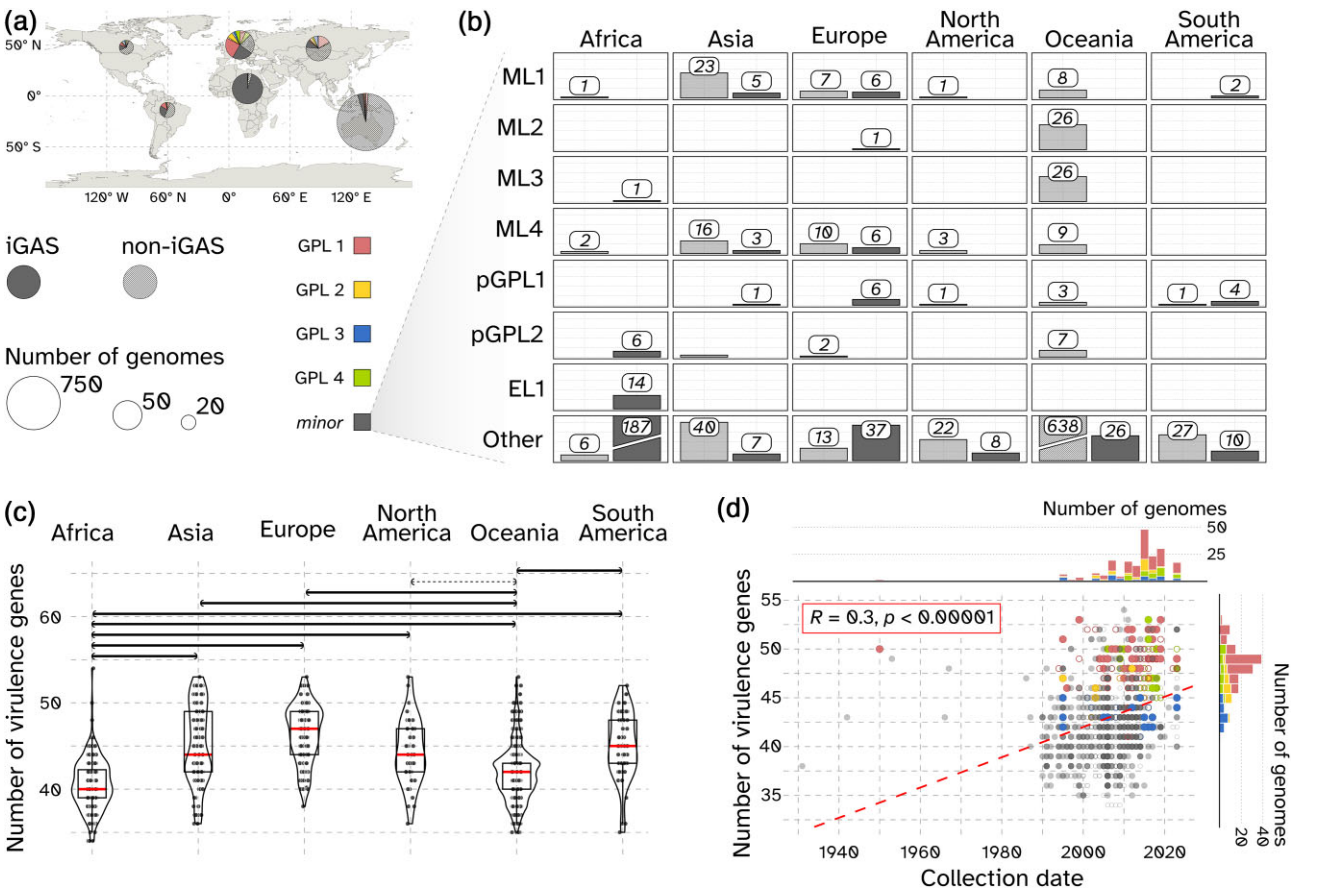

Figure 3. Geographic and temporal distribution of virulence profiles across *S. pyogenes* lineages. (A) Global map showing pie charts of lineage composition by continent. Pie-slice colors denote the four Global Pathogenic Lineages: GPL1 (ST28/*emm*1) in red, GPL2 (ST15 + ST315/*emm*3) in yellow, GPL3 (ST52/*emm*12) in blue, and GPL4 (ST39/*emm*89) in green. All other genomes remain in light gray. Solid fill indicates invasive isolates, while hatched fill denotes noninvasive isolates. Circle area is proportional to the number of genomes sampled per continent. (B) Matrix of non-GPL lineage counts by continent and infection severity. Each cell shows the count of non-iGAS (left, shaded light gray) and iGAS (right, full dark gray). The scale of *y*-axis is plotted to 50, with breaks for two out-of-scale columns, and raw counts are printed above the bar. ML: mild lineages; pGPL potentially global pathogenic lineages; and EL endemic lineages. (C) Violin plots of total virulence-gene counts stratified by continent (Africa, Asia, Europe, North America, Oceania, and South America). Individual genomes are overlaid as points; red bars mark the median. Horizontal lines indicate statistically significant pairwise differences (Games–Howell test, dotted line $P \leq .001$, full line $P \leq .00001$). (D) Scatterplot of virulence-gene count versus year of isolation for all genomes. Each point represents a genome and is colored according to its lineage identity according to the previous scheme. The dashed red line is a linear regression fit ($R = 0.30$, $P \leq .00001$). Marginal histograms show the temporal distribution of genome sampling (top) and the distribution of virulence-gene counts (right), only the GPL genomes are shown.

line of virulence genes. From a genomic surveillance perspective acknowledging the role of "founder-driven" dynamics allows for swifter detection of new relevant lineages and their clonal expansion.

Local adaptive selection and niche adaptation

Variations in the virulence repertoire can also reflect local adaptive pressures. The continents differ in host demography, climate, personal hygiene practices, preexisting immunity, HLA variability, resident microbial communities, and coinfections, hence creating a heterogeneous landscape favoring distinct virulence strategies. To validate this hypothesis, qualitative variations in the virulence genes burden were analysed. The total virulence genes burden was modeled using generalized linear models (GLMs). Preliminary dispersion checks supported the Poisson assumption; hence a Poisson GLM was fit taking into account the continent of isolation, the lineage and the isolation year. To quantify the relative contribution of the lineage compared with geographic and temporal variables, deviance reductions between nested models was computed, and a Poisson generalized linear-mixed model with lineage as a random intercept was set to estimate variance partitioning. While lineage composition explained a large fraction of virulence-gene variability (~54% of deviance reduction), continent and isolation year have a modest impact in the deviance, indicating a modest independent geographic effect after accounting for lineage. For instance, genomes sampled from North America display a higher, lineage-independent, carriage of *ndoS* gene. This gene encodes the beta-*N*-acetylglucosaminidase protein EndoS which, in non-M1T1 isolates, confers a higher resistance to neutrophils and monocytes (Sjögren et al. 2011).

Homologous recombination

GAS genomic plasticity is driven by recombination events a process, which led the emergence of pandemic lineages in Europe, as exemplified by the loss of the capsule synthesis locus *hasABC* in the *emm*89, *emm*28, and *emm*87 lineages (Turner et al. 2019). However, GAS isolates may undergo homologous recombination events with SDSE, which has a wider ecological niche and different epidemiological patterns, SDSE. Both core and accessory genes can be transferred between the two species (Xie et al. 2024b).

Bystander selection and antimicrobial usage

Antibiotic usage across the world has large spatial differences, both when comes to antibiotic consumption rates and to which antimicrobial classes are administered (Browne et al. 2021). Although in GAS MGEs rarely cocarry resistance and virulence genes, antimicrobial use can profoundly reshape the surrounding microbiota, indirectly favoring certain GAS lineages. Different antibiotics impact on competing commensals to different extents, thereby modifying ecological niches and altering the balance of circulating clones through bystander selection.

Global pathogenic lineages

To identify the most relevant GAS lineages at a global level, we selected those accounting for ≥1.5% of the 1418-genome dataset (i.e. ≥22 isolates), exhibiting an invasive infection rate above the overall average of 31.3% and showing evidence of geographic breadth: genomic clusters meeting these criteria were defined as *Global Pathogenic Lineages* (GPL). Most genomic sequences at a global level (1222/1418, 86.3%), however, were not classified as GPLs and for the sake of intelligibility are going to be named as non-GPLs clusters. Among non-GPLs, hence some clusters falling below one (or more) GPLs thresholds but with specific distributions or severity patterns will be discussed in a specific paragraph.

Most genomes from Africa (217/219, 99%) and Oceania (743/765, 97.1%) belong to non-GPLs clusters, reflecting a higher variability but also highlighting the impact of geographic sampling bias, with a skewed prevalence not reflecting the real-world epidemiology. Conversely, non-GPLs clusters play a significantly lower role in Europe (88/192, 45.8%) and Asia (96/140, 68.5%).

GPLs included GPL1 (115 genomes), GPL2 and GPL3 (28 genomes each), and GPL4 (25 genomes). A Bayesian inference-based approach was then applied to estimate GPL evolutionary rates and to construct four time-scaled, dated phylogenies (one per cluster). This was achieved by performing a genome-wide alignment (https://github.com/tseemann/snippy) using the oldest isolate within each cluster as reference, followed by detection and removal of recombinant regions. After conducting a root-to-tip linear regression analysis, an optimal root was selected to maximize the R^2 coefficient in each of the four trees. Subsequently, 10^6 Markov chain Monte Carlo iterations were run to produce the four dated phylogenies (Didelot et al. 2018). The resulting time-scaled trees were visualized using TreeViewer (Bianchini and Sánchez-Baracaldo 2024) and adjusted using the open-source software InkScape (Fig. 4, panels A–D).

GPL 1–the M1 lineages

The ST28 lineage is universally associated with the *emm1* serotype, representing the most persistent and globally dominant iGAS clone.

This lineage traces back to the late 19th century, with the most-recent common ancestor (MRCA) estimated around 1890 (Fig. 4, panel A). Early branching events during the evolution of this lineage show a deep split into two subclades, an Asian one and European one. The geographical segregation event leading to this phylogenetic split likely occurred around 1985, with a different evolutionary trajectory of the Asian subclade now characterized by a higher amount of acquired virulence and resistance genes (Fig. 4, panel A).

Over time, the European subclade underwent a stepwise succession of genetic diversification events, which lead to the emergence of a highly virulent clone (Nasser et al. 2014). Initially, an ancestral isolate of this lineage acquired a phage encoding the extracellular DNase D2 (SdaD2). Subsequently, it gained a second phage encoding the SPE A superantigen SpeA1 variant, which later evolved into SpeA2 due to a SNP in the *speA1* gene, causing a G110S substitution (Nelson et al. 1991b). Following these events, a large chromosomal region encoding several secreted toxins, including streptolysin O, was acquired via HGT (Sumby et al. 2005b, Maamary et al. 2012). Other key mutations were selected over the years, including those leading to increased production of SPE B.

The epidemiological and clinical success of *emm1* GAS started in the second half of the 20th century. The occurrence of genomic plasticity events, resulting in a different phage content, increased expression of SLO and *in vivo* regulatory gene mutations, have made this *emm*-type a leading cause of iGAS cases (Sumby et al. 2005a, Sanderson-Smith et al. 2014). This lineage has historically been identified as a leading cause of iGAS infections (Luca-Harari et al. 2008, Meisal et al. 2010), largely due to alterations in the two-component signal transduction system CovRS (Sumby et al. 2006, 2009, Chiang-Ni et al. 2009), which is implicated in virulence. However, starting in the UK, the simultaneous emergence of a novel *emm1* GAS lineage and a rise in scarlet fever notifications was observed (Lamagni et al. 2018).

The differentiation of these novel isolates from the main *emm1* GAS followed a geographical criterion and two lineages, named $M1_{global}$ and $M1_{UK}$, were defined. What set apart this novel *emm1* isolates was the consistent presence of 27 signature SNPs (Vieira et al. 2024). However, deeper phylogenetic analyses highlighted that the expansion of the $M1_{UK}$ lineage in England was just the first visible occurrence of this evolutionary trajectory. Indeed, at least two intermediate lineages named $M1_{13SNPs}$ and $M1_{23SNPs}$, carrying 13 and 23 of the 27 SNPs defining the $M1_{UK}$ lineage, respectively, were identified, but did not expand significantly in the population. The $M1_{UK}$ lineage likely emerged in 2008 (Vieira et al. 2024), and the first isolates were sampled in 2010. By 2015, it accounted for 65% of noninvasive and 84% of invasive *emm1* isolates in the UK (Lynskey et al. 2019) and, in 2020, 91.5% of *emm1* iGAS cases (Zhi et al. 2023).

As nonpharmaceutical interventions were implemented to face the initial COVID-19 pandemic waves, notifications of scarlet fever and iGAS declined in 2020. Consequently, the expansion of the $M1_{UK}$ lineage suffered a "bottleneck" effect due to reduced transmission.

In the last months of 2022 significant increases in scarlet fever and iGAS cases were reported not only in the UK (where the pediatric population was the most affected, with several fatalities) (Guy et al. 2023) but also worldwide (https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON429) (17, 43, 324–326). Notably, the $M1_{UK}$ lineage itself can be subdivided into three clades, two of which originated in the UK and have been introduced into other countries, potentially on multiple occasions (Johannesen et al. 2023, Vieira et al. 2024).

Information about the spread of the $M1_{UK}$ lineage, however, is limited to high-income countries characterized by temperate environmental conditions and no data are available regarding imported cases in low-income countries. In fact, while the name of the lineage reflects the geographic origin of the first sequenced isolates characterized by the molecular signature described above, at that time the UK was one of the few countries with specific guidelines for GAS notification and performing a genomic-based surveillance for GAS.

This implies not only that other geospatial origins for the $M1_{UK}$ lineage cannot be excluded, but also that a global epidemiology

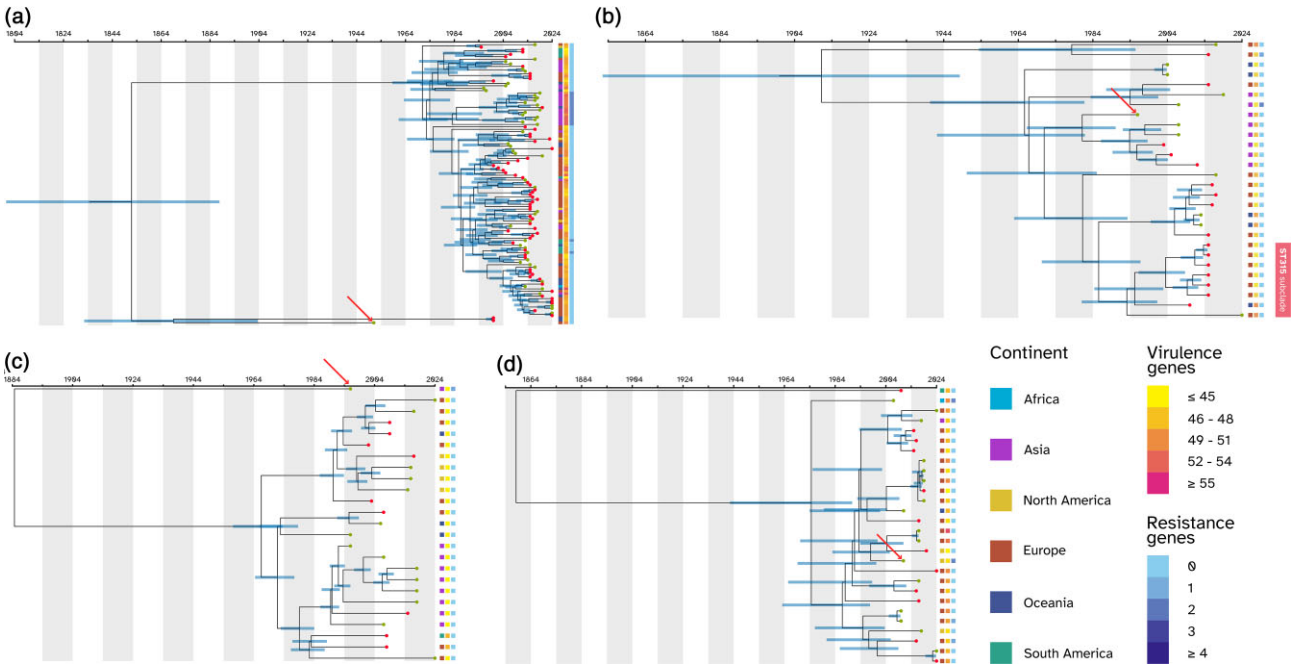

Figure 4. Time-scaled phylogenies of the four GPLs. Bayesian maximum-clade-credibility tree calibrated to collection dates along the horizontal axis: (A) GPL1 ($R^2 = 0.62$, $P \leq .00001$), (B) GPL2 ($R^2 = 0.36$, $P \leq .001$), (C) GPL3 ($R^2 = 0.73$, $P \leq .0001$), and (D) GPL4 ($R^2 = 0.37$, $P \leq .001$). Shaded gray vertical bands denote 20-year intervals; red arrows indicate the genome used as reference for single-nucleotide polymorphism mapping. Light-blue bars overlaying nodes indicate the 95% highest-posterior-density interval for each node's date estimate; tip labels are color coded according to invasiveness predicted from isolation source, red tips for invasive infections, green tips for noninvasive infections; colored squares to the right of each terminal branch denote the continent of sampling in the first column, virulence and resistance genes count are reported in the second and third columns, respectively. Metadata are color-coded according to the legend.

network, based on shared sequencing data is essential for monitoring emerging and reemerging pathogens.

Under a molecular pathogenesis point of view, the most relevant feature setting apart the $M1_{UK}$ strains from the $M1_{global}$ ones is a 5–10-fold increase in transcription of the *speA* gene (Lynskey et al. 2019, Li et al. 2023a), associated with an increased expression of the scarlet fever toxin protein SpeA. Clinically, the consequences of this molecular feature are reflected in the higher incidence of $M1_{UK}$ strains in scarlet fever cases compared with $M1_{global}$ ones (Lynskey et al. 2019). Increased *speA* transcription is evident also in the $M1_{23SNPs}$ lineage, but not in the $M1_{13SNPs}$ one. A plausible molecular mechanism underlying this phenomenon is the presence of mutations in the two-component regulatory system *covRS*, which normally suppresses virulence factors; such mutations are observed in the $M1_{23SNPs}$ and $M1_{UK}$ lineages, but not in the $M1_{global}$ and $M1_{13SNPs}$ lineages (Davies et al. 2023).

GPL 2—the STSS-causing M3 type

This genomic group overlaps with two different STs, with most genomes assigned to the ST15 and a smaller subclade belonging to the ST315 (Fig. 4, panel B). This composite lineage corresponds to the M3 serotype which, since the pregenomic era, has been pinpointed as a highly virulent GAS clone associated with high rates of iGAS and mortality (Musser et al. 1991). The first *emm*3/ST15 genome was sequenced in 2002 and drew attention due to the presence of multiple phage-mediated recombinations, which explained the unusually high virulence of this clone (Beres et al. 2002). Over the years this lineage gained the spotlight multiple times, owing to the diffusion of capsule hyperproducing strains (phenotypically characterized by a mucoid morphology) (Tamayo et al. 2010) and to multiple cases of STSS worldwide attributed to *in vivo emm*3-specific hypervirulence mutations (329, 331–334).

Moreover, large-scale epidemiological analyses highlighted the growing role of this lineage in scarlet fever-associated GAS isolates in Asia (Mu et al. 2022).

ST315, instead, is less frequently reported. In terms of MLST, the only difference with ST15 lies in the *yqiL* gene, with ST15 displaying allele 2 and ST315 allele 53. ST315 and ST15 likely shared their MRCA around 1990, when the ST315 diverged. While the clinical and epidemiological relevance of this specific subclade still needs to be fully assessed, it has been identified in multiple genomic-based epidemiology studies from Western Mediterranean European countries as a cause of iGAS infections (45, 335–337)

GPL 3—the resistant M28 type

The GPL3 overlaps with ST52, corresponding predominantly to *emm28*. Albeit this clone has been pinpointed at a global level as a frequent cause of iGAS infections (Kachroo et al. 2019, Butler et al. 2024), with a significant association between the SC1B sublineage (Kachroo et al. 2019) and pregnancy- and postpartum-related iGAS cases (Gröndahl-Yli-Hannuksela et al. 2021), this lineage is above all considered one of the most relevant in terms of antimicrobial resistance.

Specifically, ST52 has been identified in several epidemiological studies based worldwide (either based on MLST + *emm*-typing or WGS). Historically, ST52 has been one of the first lineages globally linked to macrolides resistance (341–344), as well as to "bystander selected" resistance to fluoroquinolones (Montes et al. 2010) and tetracyclines (Villalón et al. 2023). This spread of antimicrobial resistance determinants is plausibly linked to the acquisition of MGEs harboring resistance genes (Berbel et al. 2021) potentially acquired from other *Streptococcus* species, such as *S. suis* and *S. agalactiae* (Lee and Andam 2022).

In terms of geographical distribution, this lineage has been identified at a global level. Combining geographical spread and phylogenetic evolution it is possible to discern an Asian subclade, which likely diverged from the main population around 1975 and a more recent North American subclade, which emerged around 1990 (Fig. 4, panel C).

GPL 4—the acapsulated M4 type

Broadly coinciding with ST39 and *emm*4, this lineage is typically associated with infections in the pediatric population (Whitehead et al. 2011). For instance, *emm*4 isolates are a frequent cause of iGAS in children under 18 years of age (Luca-Harari et al. 2009, Guy et al. 2023, Hall et al. 2024, Ramírez de Arellano et al. 2024), are associated with ARF (Bennett et al. 2022) and carry a relevant amount of virulence genes (Fig. 4, panel D).

GPL4 is the only specific GAS lineage that consistently encodes the enzymatically active HylA hyaluronidase variant. While this feature does not allow GPL4 isolates to form a glucuronic acid/β1,3-*N*-acetylglucosamine capsule, it has been demonstrated *in vitro* that capsule production and expression in *hylA* deficient M4 strains does not enhance virulence (Henningham et al. 2014).

Analysis of MGEs and acquired resistance genes of GPLs

The set of bacterial MGEs, collectively referred to as the mobilome, plays a crucial role in *S. pyogenes* genome evolution. To evaluate the contribution of MGEs to genomic diversity within the GPL framework, a mobilome analysis was performed on reference genomes from each GPL cluster (including 24, 2, 7, and 11 genomes for GPL1, GPL2, GPL3, and GPL4, respectively). Reference genomes were selected based on the availability of complete genome sequences. The mobilome of complete genomes was analysed assessing the presence of ICEs and MEs in the genomes with ICEfinder v1.0 (https://bioinfo-mml.sjtu.edu.cn/ICEfinder/index.php), the presence of prophages with PHASTEST v3.0 (Wishart et al. 2023), and the integration sites of prophages, ICEs/IME, DNA sequence analysis was performed with Artemis/ACT v17.0.1 (Carver et al. 2012, Colombini et al. 2023). Manual annotation of MGEs was carried out by blast homology searches of the databases available at the National Center for Biotechnology Information (NCBI) (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins, accessed on March 2025), and the Pfam protein family database (available under the InterPro consortium, https://www.ebi.ac.uk/interpro/search/sequence/, accessed on March 2025).

The analysed mobilome accounted for 2.5%–12.5% of the *S. pyogenes* genomes and included a total of 30 distinct prophages, 2 ICEs, and 1 IME. All isolates carried between 2 and 6 prophages, with GPL3 isolates also harboring 2 ICEs/IMEs and strain HKU419 (GPL1) carrying an ICE.

ICEs are capable of intracellular transposition to a new genomic site or intercellular transposition via conjugation (Burrus and Waldor 2004). In contrast, IMEs are capable of self-intracellular transposition but rely on the conjugation machinery of a helper ICE or conjugative plasmid for intercellular transfer (Guédon et al. 2022). Both ICEs and IMEs exhibit a typical modular organization consisting of (i) a mobilization module for intercellular transposition, (ii) an integration/excision module for intracellular transposition, and (iii) an adaptation module containing cargo genes that may confer a fitness advantage to their bacterial host. In *S. pyogenes*, ICE-associated cargo genes frequently include antimicrobial resistance determinants, such as *ant(6)-la*, *aph(3')-III*, *erm*(A), *erm*(B), *erm*(T), *erm*(TR), *mef*(A), *msr*(D), *tet*(M), and *tet*(O) conferring resistance to aminoglycosides, macrolides, and tetracyclines (358–360). Additionally, ICEs can be transferred among streptococcal species and can recombine or integrate into other MGEs like prophages, forming composite genetic structures (Iannelli et al. 2014, Sanderson-Smith et al. 2014). The ICEs/IMEs found in the *S. pyogenes* complete genomes of the GPL clusters were previously identified (Arcari et al. 2025) and included the 65.2-kb composite element GAS-ICE18.1 (strain HKU419, GPL1) composed of the *S. pneumoniae* transposon Tn*6003* (GenBank number AM410044.5) carrying four resistance determinants (*tet*(M), two copies of the *erm*(B) gene, and *aph(3')-III*) integrated into the backbone of a *Streptococcus anginosus* ICE*San49.2*-like element (PP062800.1).

Streptococcus pyogenes prophages encode a number of potential virulence factors including superantigens (*speA*, *speC*, *speG*, *speH*, *speI*, *speK*, *speL*, and *ssa*), DNases (*spd1*, *mf2*, *mf3*, and *mf4*), phospholipase A2 (*sla*), and the macrolide resistance gene pair *mef*(A)/*msr*(D) (Suvorov et al. 2009, Iannelli et al. 2018). Prophages of GPL clusters ranged in size from 13 389 bp (*S. pyogenes* phage 6180.4) up to 45 960 bp (*S. pyogenes* phage 6180.1) and included 27 characterized prophages, of which five were satellite prophages. Prophages are responsible for the different distribution of superantigens within the GPL clusters. The streptococcal superantigen *ssa* gene was found only in prophages of GPL2 and GPL4, consistent with its known association with *emm3* (Reda et al. 1994) and *emm4* (van der Putten et al. 2023) strains. Similarly, the *speA*, *speI*, and *speJ* genes were found only in prophages of GPL1, in agreement with previous reports linking these prophage-carried superantigens to *emm1* strains (Chang et al. 2011a, Rantala et al. 2012). Conversely, *speG*, which is not univocally associated with prophages, was found in all prophages except those associated to GPL4.

MGEs integration into bacterial genomes occurs via homologous recombination between a sequence located on the MGE (*attI* or *attP*) and an attachment sequence present in the bacterial chromosome (*attB*). MGEs integration in the *S. pyogenes* chromosome mainly targets conserved genes such as tRNAs or tmRNAs. Integration results in the duplication of attachment sequences (ranging from 12 to 96 bp), generally restoring the functional coding sequence (CDS). In some cases, however, integration occurs within intragenic regions (Fouts 2006). MGEs of the GPL clusters were found integrated at 16 chromosomal integration sites (15 within CDS and one intergenic region), some of which were shared between MGEs of different GPL clusters (Fig. 5). Among the 16 integration sites, 7 contained only MGEs from a single specific GPL, while the remaining sites harbored elements from two or three GPLs. No integration sites were found to contain MGEs from all four GPLs.

Mobilome analysis was extended to all 196 genome sequences included in the four GPL clusters (115 for GPL1, 28 for GPL2, 28 for GPL3, and 25 for GPL4) regardless of the assembly state. Gene presence in the single genomes was assessed using Roary.

The mobilome of most GPL1 isolates contains prophages 5005.1, 5005.2, and 5005.3 (detected in 113, 103, and 101 isolates respectively), as reported for many strains causing disease since the mid-1980s (Nasser et al. 2014), while a subset of eight isolates contains prophage 315.3. The composite ICE-GAS18.1 was found in 14 genomes, while Φ1207.3 and Φ1380.vir were detected in two isolates. The latter is homologous to the Hong Kong scarlet fever-associated prophage ΦHKU488.vir (Ben Zakour et al. 2015), which was identified in 14 genomes. Finally,, prophage Javan 488

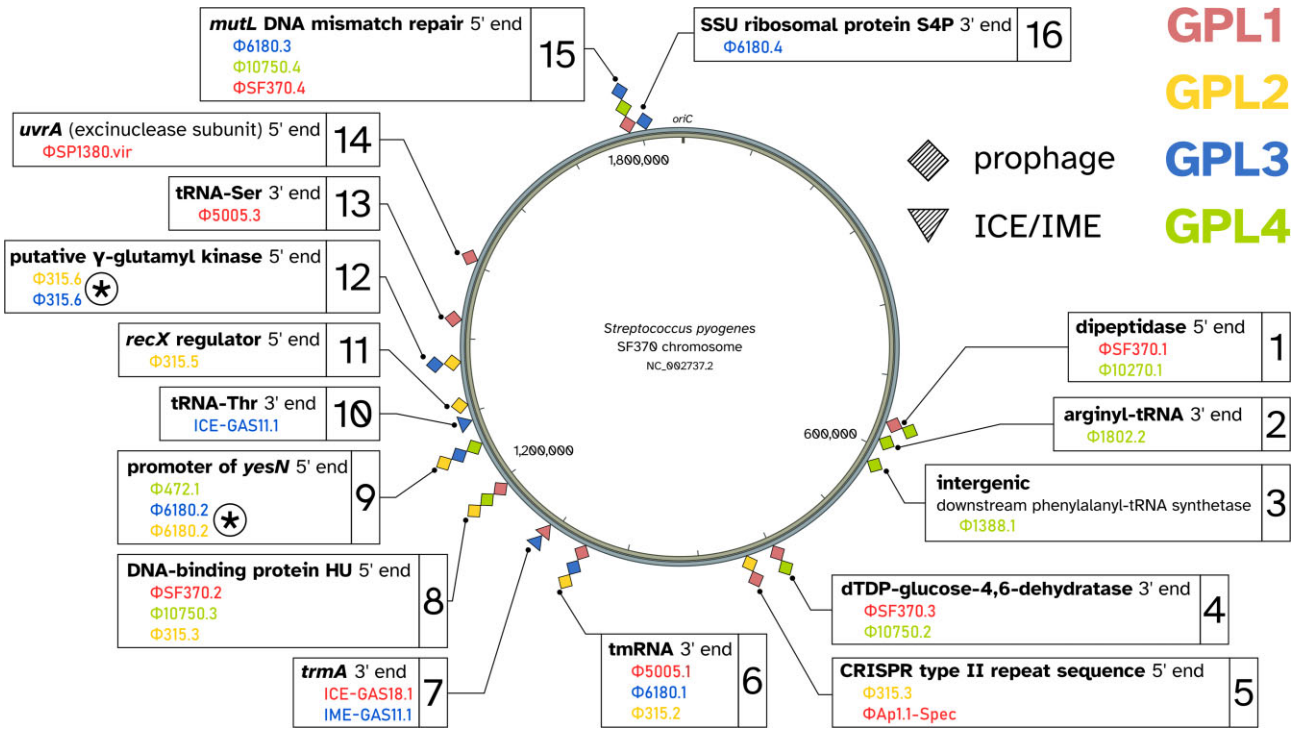

Figure 5. Integration sites of MGEs in the *S. pyogenes* chromosome of GAS strains belonging to GPLs. Analysis of the chromosomal hotspots for MGEs integration in 44 *S. pyogenes* complete genomes belonging to the GPLs (24 genomes for GPL1, 2 for GPL2, 7 for GPL3, and 11 for GPL4), available in the NCBI database (last accessed February 2025). *Streptococcus pyogenes* chromosome is represented by a circle, and the origin of replication (*oriC*) is indicated; nucleotides reported on the map refer to the SF370 M1 strain used as a reference. Diamonds and triangles around the circle indicate the insertion sites of prophages and Integrative Conjugative/Mobilizable Elements (ICEs/IMEs), respectively. Elements belonging to the strains within the same genomic cluster are depicted with symbols of the same color, and the name of one representative MGE per cluster is reported. Asterisks denote shared MGEs between different GPLs.

was found in 10 genomes, while prophages Javan 487, 489, 490, 506, ΦGAS2.1, and ΦAp1.1-Spec were each detected in only one isolate. Importantly, several of these prophages including Javan 488, Javan 506, Φ1380.vir, ΦHKU488.vir, and ΦAp1.1-Spec carry the *speC* gene, which encodes the SPE C.

Mobilome of most GPL2 isolates contains prophages 315.1, 315.2, 315.3, 315.5, and 315.6 (24, 24, 25, 21, and 23 isolates, respectively) of the M3 representative MGAS315 strain (Beres et al. 2002). A subset of 11 isolates contains ΦNIH1.1, while one strain contains Φ1207.3.

Mobilome of most GPL3 isolates contains prophages 6180.1, 6180.3, and 6180.4 (21, 22, and 25 isolates, respectively), of M28 reference MGAS6180 strain, while a minority contains 6180.2, 315.6, 5005.1, and ΦGAS5.1 (5, 2, 2, and 1 isolate, respectively). Additionally, ICE-GAS11.1 was present in 19 isolates, while IME-GAS11.1 in 24 isolates. Moreover ICE-SHemm28 was detected in one isolate.

Almost all GPL4 isolates contain prophages Javan 493, 10750.1, 10750.2, 10750.3, 10750.4, and 5005.2 (16, 15, 16, 16, 15, and 19 genomes, respectively). Four isolates contain Φ1802.2, while one isolate contains ΦGAS5.1 and one isolate carries a putative ICE containing the *tet*(O) resistance determinant.

This variability underscores the genomic plasticity among strains of the same GPL. Even within a single cluster, integrated prophage content can differ significantly, with distinct prophages inserted at different genomic sites still encoding identical virulence factors. Moreover, ICEs can be present in a subset of strains, suggesting that their acquisition can be relatively recent, possibly selected by selective pressure from antimicrobial therapy.

Relevant, "non-GPL" clusters

The use of genomic data to reconstruct the global epidemiology of a pathogen is not a bias-free approach. First, different areas have different distribution of sequencing technologies, creating a bias in data distribution. Second, it is possible to observe diverging trends of GAS clinical manifestations correlated with economic inequities (Avire et al. 2021): socially disadvantaged and indigenous populations suffer disproportionately high rates of iGAS disease, and within the same city, low-income districts harbor a greater repertoire of *emm* types compared to high-income areas, driven by increased transmission opportunities and frequent HGT events (Tartof et al. 2010).

Hence, several clusters that fall below one or more GPL thresholds still deserve close attention. Specifically, it was possible to identify endemic pathogenic lineages (causing iGAS with a high prevalence, but geographically limited, named EPL), mild lineages (highly represented, but rarely causing iGAS, named ML), and potential GPLs (causing iGAS with a high prevalence and widely spread, but composed of less than 22, named pGPL)

Mild lineages

- ML1 is composed of 56 genomes, 13 of which (23%) from isolates causing iGAS, and coincides with ST36. This group has been historically associated with *emm*12, encoding a variant typically causing pharyngitis (Smeesters et al. 2024). GAS isolates characterized by *emm*12 are the second most common group both in Europe (Hall et al. 2024) and in the USA (Shulman et al. 2009). Despite this tight association with *emm*12, a subset of ST36 isolates belonging to minor clade acquired

a new M protein following a genetic recombination, hence defining a new *emm*82/ST36 group (Unoarumhi et al. 2023).

- ML2 is composed of 27 genomes, one of which (4%) from an isolate sampled in a soft tissue invasive infection, and broadly coincides with ST1032 (22 genomes) and ST191 (5 genomes). Information on these STs in the scientific literature is scarce and there is not any documented association with specific *emm* types in epidemiological surveys to date. An *in silico emm* prediction (https://github.com/MDU-PHL/emmtyper) identified the *emm*25 allele (a not-so-common one) in all ML2 genomes. Importantly, this lineage also displays a highly specific geographic association: all ST1032 isolates have been sampled from the skin of individuals living in the Fiji Islands, whereas ST191 isolates have been sampled from throat swabs in Australia and New Zealand. Notably, the one isolate causing an iGAS infection is the only one sampled outside Oceania (Sweden) and the only representative of ST660.
- ML3 is composed of 27 genomes, one of which (4%) from an isolate sampled in a soft tissue invasive infection, and coincides with ST10 (24 genomes), ST1003 and ST1063 (2 and 1 genomes, respectively). While ST10 has been notably reported as the genetic background of an *emm*93, emergent, multidrug-resistant clone in Israel, the respective genomes were not uploaded as RefSeq sequences and were not included in the analyses (Ron et al. 2022). Analysed ML3 genomes, instead, displayed remarkable similarities with ML2 in terms of geographic distribution: all ST10 and ST1003 isolates have been sampled from the skin or pharyngeal swabs of individuals living in Oceania (Fiji Islands, Australia and New Zealand) and the *in silico emm* prediction (https://github.com/MDU-PHL/emmtyper) identified the *emm*70 allele. The single ST1063 isolate, instead, encoded a different M variant (*emm*93 allele) and, similarly to ML2, it was the one causing an iGAS infection and the only one sampled outside Oceania as well (Kenya).
- ML4 is composed of 46 genomes, 6 of which (13%) from isolates causing iGAS, coincides with ST101 (26 genomes), ST646 (16 genomes), ST142, and ST407 (3 and 1 genomes, respectively) and is associated with the *emm*89 allele. This cluster, together with ML1, is the most relevant causes of non-iGAS infections both Europe and North America over the past decade (356, 390–393), while in Asia it is well represented in Japan (Ikebe et al. 2021). Notably, after major recombination events, an acapsular and high-toxin-expressing variant of *emm*89 was gaining a stable spot in the UK epidemiology simultaneously with the development of the $M1_{UK}$ lineage (Turner et al. 2015a). Conversely, in Japan the spread of *emm*89 isolates displays a double nature: on one hand it is a major cause of iGAS (mostly STSS) (Ikebe et al. 2021) while, on the other hand, endemic virulence of this clone is curbed by a SNP in the ferrichrome transport system permease *fhuB* limiting *S. pyogenes* growth rate in human blood (Ono et al. 2025). ST646 isolates, instead, have recently been described as the cause of a major iGAS outbreak in frail patients hospitalized in a head and neck surgical oncology ward (Hayama et al. 2024).

Potential GPLs

- pGPL1 is composed of 16 genomes, 9 of which (56%) are associated with invasive infections, coincides with ST46 and is associated with the *emm*22 allele. Generally defined by resistance to tetracyclines mediated by *tet*(M) and to MLS_B, mediated by *erm*(A) and/or *erm*(B) (Pérez-Trallero et al. 2007), this cluster has recently been identified as cause of necrotizing fasciitis and STSS cases in China (Zhang et al. 2024). This lineage is acapsular (owing to the absence of the entire *hasABC* locus) and high-toxin-expressing (Flores et al. 2019). While its distribution in western countries is scarce it is on the rise: in the UK it represented <0.5% of all sequenced isolates in the 2016–2017 period; in 2022–2023 (after the COVID-19 pandemics and concurrently with the peak of the $M1_{UK}$ lineage spread) instead, *emm*22 isolates represented 5.9% of all GAS (Hall et al. 2024). Notably, out of the 16 genomes belonging to pGPL1 in our dataset, four were sampled in Brazil from 2011 to 2013 as causative agents of scarlet fever. Owing to its frequent association with MLS_B resistance and the high pathogenic potential, this lineage has the potential to become a major epidemic clone in the future;
- pGPL2 is composed of 16 genomes, 6 of which (37.5%) are associated with invasive infections, coincides with ST178 and is associated with the *emm*44 allele. Infections caused by pGPL2 isolates potentially have a link with socio-economic status, being identified as a major iGAS cause in drug users and homeless persons in France (Cady et al. 2011, Soriano et al. 2014) and as a relevant pathogen in postpartum infections (both in the newborns and in women) in LMICs (*S. pyogenes in Neonates and Postpartum Women:…*). Additionally, *emm*44 isolates are associated with tetracycline and MLS_B coresistance owing to the carriage of the *tet*(M) and *erm*(B) genes in Tn916-like elements.

Endemic pathogenic lineages

- EL1 is composed of 14 genomes all associated with invasive infections, coincides with ST450 and is associated with the emm166.4 variant allele. Despite the high invasive rate, all isolates were sampled in Kenya (specifically in Kilifi) between 1998 and 2011. During this period multiple clonal groups were identified, but ST450 was the one linked to rapid expansions and to multiple cases of pediatric pneumonia and bacteremia (Seale et al. 2016).

Conclusions and perspectives

Integrating genomic data with rich contextual metadata is critical for transforming raw sequence information into actionable insights. Genomes lacking metadata, such as collection date, geographic location, host demographics, and clinical manifestation, have limited epidemiological value. Sequence data alone cannot support accurate reconstruction of transmission chains, temporal dynamics, or risk-factor analyses (Aarestrup et al. 2012). Conversely, the risk of relying exclusively on local metadata without developing global genomic frameworks is to overlook broader evolutionary trends, intercontinental introductions, or the emergence of novel high-risk clones. For example, retrospective metadata alone might reveal an increase in pharyngitis cases in a single city, but without comparative genomics, it would be impossible to determine whether this reflects local expansion of a known lineage or the incursion of an exotic clone with unique virulence determinants (Sharma et al. 2019). Therefore, integration and curation of metadata in sequence repositories is essential: clinical, environmental, historical, and geographic information, just to list the main ones, should accompany every genome submission to enable large-scale correlational studies and risk modeling (Bonomo et al. 2025).

NGS has revolutionized our ability to generate high-resolution snapshots of bacterial populations in real time, and GAS is no exception. As of today, it is possible to perform phylogenetic cluster-

ing, outbreak detection, and fine-scale tracking of virulence- and resistance-genes spread as they happen.

For the clinical microbiologist of the near future the real-time integration of genomic surveillance into routine laboratory practice will become a reality, with pipelines that automatically couple sequence data to electronic health records and regional public-health databases. With the implementation of a routinary NGS-based epidemiology, it will be possible to directly guide outbreak interventions and infection-control measures (Bergin et al. 2018), potentially performing *in silico* genotyping to predict antimicrobial susceptibility or toxin profiles, thereby enabling tailored therapy (Metzgar et al. 2009).

NGS data and metadata, moreover, entails other information and applications besides epidemiology: in the context of vaccine discovery, global genomic datasets enriched with information on patient age, disease severity, and geographical origin allow identification of conserved antigenic targets and assessment of their population coverage, as demonstrated in recent proposals for multivalent M-protein and non-M-protein vaccines (Walker et al. 2014, Davies et al. 2019).

Conflict of interest

None declared.

Funding

The writing of this review was supported by EU funding within the MUR PNRR Extended Partnership Initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT, "One Health Basic and Translational Research Actions addressing Unmet Needs on Emerging Infectious Diseases", Spoke 3, WP1) and by the Rapid prediction and diagnosis of SEPsis in Solid Organ Transplant: The Italian national initiative for an interactive risk-stratification and Cost-optimization Online DatabasE, SEPSOT-CODE (ID N. S3. P0002 – CUP J33C23004160006 - CODSOG_000038).

Supplementary data

Supplementary data is available at *FEMSRE Journal* online.

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Received 1 July 2025; **revised** 5 November 2025; **accepted** 5 November 2025