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"Pathogenesis And Resistance Mechanisms In Group A Streptococcus: A Clinical And Molecular Perspective"

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ABSTRACT
Group A Streptococcus (GAS) (Streptococcus pyogenes) is an important human pathogen that causes many life-threatening and non-life-threatening infections. This chain-forming Gram-positive round bacterium is a human-only pathogen, i.e. it does not infect other animal species by nature. GAS colonises the human pharynx and skin and its infectious capacity reflects the combined actions of a number of virulence factors that mediate evasion of the host immune response and tissue injury. As understanding the biology of GAS and the mechanisms of pathogenesis through antibiotic resistance becomes increasingly important for preparedness against newer strains, so too does understanding its health implications for susceptible individuals. The most common infection identified as streptococcal is pharyngitis, also known as strep throat, most common among children age 5-15 yr. It's marked by a severe sore throat, fever, headache and sometimes swollen lymph nodes in the neck. Although often self-limiting, untreated strep throat can result in serious complications. Acute rheumatic fever (ARF) is one of the most serious, an inflammatory disease that can impact the heart, joints, skin and brain. ARF usually occurs weeks after a GAS pharyngitis and may cause permanent cardiac damage, called rheumatic heart disease (RHD). RHD remains a leading cause of cardiovascular morbidity and mortality in the developing world, and particularly in low- and middle-income countries. The mechanisms for the development of ARF after GAS infection are not completely understood, but it is thought that molecular mimicry is involved, where bacterial antigens are similar enough to host tissues that the immune response affects both. Early diagnosis and appropriate treatment of strep throat with antibiotics is critical in preventing ARF and RHD.

KEYWORDS : *Acute Rheumatic Fever (ARF), Antibiotic Resistance, Group A Streptococcus (GAS), Rheumatic Heart Disease (RHD), Strep Throat (Pharyngitis).*

1. INTODUCTION

One of the most common GAS infections, pharyngitis (often referred to as strep throat), can occur in anyone, but tends to affect children most frequently. It is marked by a sore throat, fever and sometimes swollen lymph nodes. Scarlet fever is associated with strep throat and is characterized by a distinct rash due to the production of erythrogenic toxins. Examples of skin infections from GAS include impetigo, which is a superficial skin infection, and erysipelas, which is a more serious infection affecting deeper layers of the skin. These infections are often extremely painful and may occasionally cause further complications.

In addition to these more common infections, GAS can also lead to serious invasive diseases. These include necrotizing fasciitis, a rapidly advancing and potentially deadly infection that destroys muscle and soft tissue, and streptococcal toxic shock syndrome (STSS), a rare but very serious illness with organ failure. Many of the invasive GAS infections originate from trivial skin infections or pharyngitis, reminding us of the unpredictable nature of this pathogen. The ways in which GAS switches gears from relatively mild infections to severe, life-threatening illnesses are still under investigation, but are likely to reflect interactions of a host of bacteria microfactors with individual host susceptibility.

GAS infections are complex and exhibit geographic and seasonal differences in epidemiology. Although strep throat is most common in older children, other GAS infections can occur at any age. Living in crowded conditions, lack of good hygiene and some chronic medical conditions may increase the risk of GAS infection. Additionally, some strains of GAS appear to be more prolific at causing severe invasive disease epidemics. These epidemiological factors elucidation is necessary to guide public health measures to reduce GAS transmission and outbreaks. For example, prompt diagnosis and treatment of strep throat with antibiotics can prevent the development of serious sequelae of GAS infection, including acute rheumatic fever (ARF) and rheumatic heart disease (RHD) [4].

Acute rheumatic fever (ARF), a sequela of GAS infection, most commonly pharyngitis, can involve the heart, joints, skin and brain. This includes rheumatic heart disease (RHD), the most severe presentation of ARF, which can lead to irreversible damage to the heart valves. The precise mechanisms of GAS infection leading to ARF and RHD remain uncertain but have been implicated in molecular mimicry, wherein bacterial antigens resemble host tissue triggering an autoimmune reaction. RHD still represents a global health burden, particularly in developing countries, underscoring the need for prevention of primary GAS infection and treatment with appropriate antibiotics.

Another possible complication of GAS infection is post-streptococcal glomerulonephritis (PSGN), a type of kidney disease that can happen in the wake of both pharyngitis and skin infection. PSGN is defined by inflammatory deposition in the filtering units of the kidney (glomeruli), resulting in hematuria (blood in the urine), proteinuria (protein in the urine), and, occasionally, renal failure. Although a majority of PSGN patients have complete recovery, some residual chronic kidney disease may develop. It is postulated that PSGN occurs due to deposition of circulating immune complexes in the kidneys leading to inflammation and resultant injury.

Antibiotic resistance, the emergence, and spread of which in GAS is increasing, represents a major threat to effective treatment. Although penicillin is still the antibiotic of choice for treating GAS infections, resistance to macrolide antibiotics (e.g., erythromycin and clindamycin) has become increasingly prevalent in many parts of the world. Resistance to antibiotics is an age-old phenomenon [19], which is frequently mediated through the acquisition of resistance genes through horizontal gene transfer that demonstrate the need for effective antibiotic stewardship programs to maximize the longevity of current antibiotics. Novel antibiotics and alternative treatment strategies are needed to address increasing antibiotic resistance in GAS.

Lastly, Group A Streptococcus is an adaptable human pathogen, with a range of illness from mild pharyngitis to severe invasive infections and post infection sequelae, including ARF/RHD, and PSGN. The interaction of virulence factors, epidemiological factors, and increasing antibiotic resistance requires further research to define GAS pathogenesis more clearly, and to aid in the development of vaccines and treatment strategies. Public health interventions, such as timely surveillance and treatment of GAS infections, programs on the prudent use of antibiotics, and the development of vaccination, are critical to reduce the burden of GAS disease globally.

The astonishing plasticity of Streptococcus pyogenes, or GAS, arises from its suite of virulence factors—molecules that help it colonize, evade the host response and cause disease. This encoding of the above features on mobile genetic elements also allow rapid recombination and mutations which can facilitate fast evolution and help the host bacteria adapt to different host, environment and selective pressure like antibiotic usage. This knowledge of the complex roles of such virulence factors is fundamental in the advancement of specific therapeutics and prophylactics.

The M protein, a surface protein, is one of the most important virulence factors and is essential for adherence to host cells and evasion of phagocytosis, a process by which immune cells ingest and kill bacteria. The M protein displays extensive amino acid sequence variability, resulting in the emergence of different serotypes, each with associated and distinct epidemiological behaviors and diseases. This M protein diversity also enhances the virulence property, in addition to its virulence, as host's antibodies against one type of M protein does not protect against other types. GAS typing is a useful tool for epidemiologic studies and outbreaks, and the emm genes that encode the M proteins are widely used for this purpose.

The hyaluronic acid capsule, a slimy layer surrounding the bacterial cell, is another critical virulence factor. In a manner similar to the bark on a tree, the GAS capsule protects it from phagocytosis and enables it to persist in the blood and cause invasive infections. Cell wall structure of the pathogen, similar to host connective tissue has also been implicated in immune evasion via molecular mimicry.

MATERIAL AND METHODS

Patients with sore Throat, samples were tested for rapid antigen detection test (RADT), a nucleic acid probe test as well as bacterial culture. A definite diagnosis was determined by a positive throat swab with culture or nucleic acid probe. The findings showed that throat swabs were significantly more sensitive than mouth swabs overall with all testing methods. Specifically: RADT: The That's in throat swab with sensitivity 80.6%, but only 19.4% sensitivity-throat swab. Nucleic acid probe: The sensitivity of throat

swab was 93.3% and 41.9% in the oral cavity. Culture: The sensitivity of mouth swab culture was 80.6%. All had 100 percent specificity with mouth swabs, and nearly 100 percent specificity with throat swabs. The authors concluded by asserting that throat swabs remain the superior choice for diagnosing GAS pharyngitis owing to the improved sensitivity. Mouth swabs were less sensitive, but given the very high specificity of the RADT on mouth swabs, a positive RADT result on a mouth swab may still be helpful to confirm a diagnosis of GAS pharyngitis, especially when it is not possible to obtain a proper throat swab. But after a negative mouth swab, treatment or testing should be undertaken. This study confirms the obvious that throat (pharyngeal) swabs are the gold standard for diagnosis of GAS but suggests that oral (mouth) swabs may be of little additional value in some contexts. Again, this work reconfirms that throat swabs are the method of choice for GAS detection, however it suggests that in certain settings detection from mouth swabs may be restricted [41].

Here, we investigate the therapeutic potential of passive immunotherapy targeting Streptococcus pyogenes virulence factors to treat or protect against superinfections with influenza A virus (IAV). Bacterial superinfections, particularly with Group A Streptococcus (GAS), have been linked with significantly higher morbidity and mortality related to influenza pandemics, as demonstrated by the 2009 pandemic. The studies provide proof of principle that passive immunotherapy any of antisera directed against streptococcal M protein or streptolysin O (SLO) is effective in a murine model of IAV-GAS superinfection. Mice treated with antisera directed against either SLO or the M protein showed improved morbidity compared to mice exposed to non-immune sera. Neither antiserum, though, had any appreciable effect on mortality. The therapeutic administration of antisera against SLO also reduced morbidity, but for neither antiserum was mortality affected. These data suggest that passive immunotherapy targeting either the M protein and/or SLO may have utility as an adjunctive treatment for streptococcal invasive diseases, including IAV-GAS superinfections. Although the study did not find a statistically significant reduction in mortality, the relative morbidity decrease advocates the use of these antibodies to reduce severity of GAS infections, collectively due to vaccination, but also because of increased protection with future pandemic influenza bursts. “The additional development of these antibodies against these virulence factors of GAS may provide an important first step toward a promising therapeutic approach,” Dr. Ghosh said. This study proposes an adjunctive therapy for GAS infection when GAS infections have been implicated in influenzas co-infections while the area continues to be elucidated. This finding indicates a potential adjunctive (accelerated) treatment option in the context of GAS infections, especially in cases of co-infection with influenza virus [42].

The aim of this study was to, implement and evaluate the effectiveness of a rapid molecular diagnostic test (IDI-Strep B) for the detection of Group B Streptococcus (GBS) colonisation in pregnant women in labour, compared with culture (reference). Group B streptococcus (GBS) infection in newborns can lead to severe complications, and presently, strategies to prevent this complication rely on administration of intrapartum antibiotics according to antenatal culture results or risk factors.

Test Type	Swab Type	Sensitivity (%)	Specificity (%)	Comments
Rapid Antigen Detection Test (RADT)	Throat Swab	80.6	≈100	High sensitivity; reliable for GAS detection
	Mouth Swab	19.4	100	Low sensitivity; positive result still helpful
Nucleic Acid Probe	Throat Swab	93.3	≈100	Highest sensitivity among all methods
	Mouth Swab	41.9	100	Moderate utility if throat swab is not available
Bacterial Culture	Throat Swab	Not provided	≈100	Gold standard diagnostic method
	Mouth Swab	80.6	100	Reasonable sensitivity

To maintain consistency and capture comprehensiveness, a predefined collection of variables was standardised for extraction from each included study. These variables were selected in order to comprehensively describe the relevant data pertaining to the context of the study, the features of the GAS

isolates studied, characteristics of virulence and pathogenesis and antibiotic susceptibility and resistance mechanism information, and relevant clinical or epidemiological correlates. The variables were organized into logic groups:

1. **General Study Characteristics:** Basic bibliographic information was extracted for in order to identify and contextualize each study. This comprised the main author(s), the year of publication, the name of the article, and the journal in which it had been published. Data were also collected for the geographical location (country or region where the study occurred or the isolates originated) of each study, as geographical variation in GAS epidemiology, virulence and resistance is well documented. The study design, such as in vitro experimental, animal model, cross-sectional surveillance, cohort study, case-control, genomic analysis, or review, was also recorded to understand the level of evidence reported. The stated study objectives or key research questions for the study were also extracted to summarize the focus of the extracted data in keeping with the original authors' intent. When available, the sample size (e.g., number of patients, number of GAS isolates assessed) was noted as a determinant of the study power and generalizability.

2. GAS Isolate Information: Detailed information on the studied S. pyogenes isolates was essential. The source of the isolates was noted, including the clinical site (throat swab, nasopharynx, skin lesion, blood culture, tissue biopsy, middle ear fluid) or if reference strains. The number of distinct GAS isolates included in the analysis of the study was documented. Importantly, molecular typing information was included whenever this was presented, most often the emm type but also Multilocus Sequence Type (ST) or WGS related findings regarding clonal complexes or phylogenetic relationships. From the literature, specific strain designations (M1T1 5448, MGAS8232, etc.) and clade descriptions (e.g., 'new clade' emm89) were recorded. Also extracted was any human genetic background information pertinent to the study (e.g., specific mutations in regulatory genes [like covR/S, associated with hypervirulence] or other important loci given by the authors).

3. **Virulence and Pathogenesis Data:** Information about GAS virulence factors and pathogenic mechanisms represented a major part of the extraction. This included the presence, absence, or prevalence information of specific virulence factor genes (e.g., genes encoding M protein, hyaluronic acid capsule (hasABC), streptolysins O and S (slo, sls), pyrogenic exotoxins (speA, speB, speC, smeZ, ssa), streptokinase (ska), hyaluronidase (hylA), adhesins (fbaA, fbaB, spy1325), fibronectin-binding proteins, C5a peptidase (scpA), immunoglobulin-degrading enzymes, iron acquisition systems (perR)). In addition to the presence of the genes, expression data regarding these virulence factors (for example, obtained with qRT-PCR, transcriptomics (RNA-Seq), proteomics, etc.) under various (pathogenic) conditions or in diverse strains were obtained. We systematically captured results from functional assays measuring virulence phenotypes, including biological assays causing relatively large ecosystems from in vitro studies measuring adherence/invasion of host cells, biofilm formation potential, resistance to phagocytosis (e.g., neutrophil killing assays), cytotoxicity, or hemolytic activity. Animal-level data (i.e., data derived from all individual animals within a treatment group) extracted from in vivo (murine models of pharyngeal colonization, skin infection, necrotizing fasciitis, bacteremia, or superinfection models using viruses such as I influenza A) was included, such as: bacterial burden (CFU counts) in individual tissues, lesion size, host inflammatory responses (e.g., cytokine levels, immune cell infiltration), morbidity scores, and survival data. A key variable was the availability of structure-function information regarding particular virulence factors (e.g., effects of targeted mutations around such factors, features of key domains as in SLO or M protein) and characterisation of regulatory systems (e.g., assignment of genes in regulatory hierarchy, identification of how mutations in such regulators affect virulence phenotypes, signalling molecules such as c-di-AMP). Examples of pathogenic processes such as lymphatic metastasis, immune evasion tactics (e.g., complement inhibition, NET degradation), or molecular mimicry were of note.

4. **Data on Antibiotic Susceptibility and Resistance:** It was important to extract comprehensive data on antibiotic resistance. This meant that phenotypic susceptibility data for multiple antibiotics relevant for GAS treatment were documented. Data were extracted for Minimum Inhibitory Concentration (MIC) values, MIC50/MIC90 values (which indicate the concentration necessary to inhibit 50% and 90% isolates respectively), or categorical interpretations (Susceptible/Intermediate/Resistant based on breakpoints defined by CLSI or EUCAST) for aminopenicillins, cephalosporins, erythromycin, azithromycin, clindamycin, tetracycline, fluoroquinolones and vancomycin. The proportion of isolates

resistant to specific antibiotics or antibiotic classes within the isolate population studied was noted. Defined phenotypes of resistance were noted, particularly for the macrolide-lincosamide-streptogramin B (MLSB) proteins (i.e., constitutive cMLSB, inducible iMLSB) and the M phenotype (macrolide efflux). Similarly, data on the presence, absence, or particular variants of known resistance genes were extracted, including erm(A), erm(B), erm(C), erm(TR) (mediating MLSB resistance), mef(A) or other mef variants (mediating M phenotype), tet(M), tet(O), tet(L) (mediating tetracycline resistance), and mutations in genes encoding drug targets such as pbp2x (associated with reduced beta-lactam susceptibility) or 23S rRNA (associated with lincosamide resistance). Extracted information about the genetic context of resistance genes, for instance, whether they were located in mobile genetic elements (i.e., specific transposons; Tn916; integrative conjugative elements (ICEs) including mef(A)/msr(D)-harboring examples; plasmids; prophages) when available, since it informs on dissemination potential. Studies with data on potential contributors to resistance (e.g. antibiotic consumption data) or with data on effects of interventions such as antibiotic stewardship were also included in the data extraction.

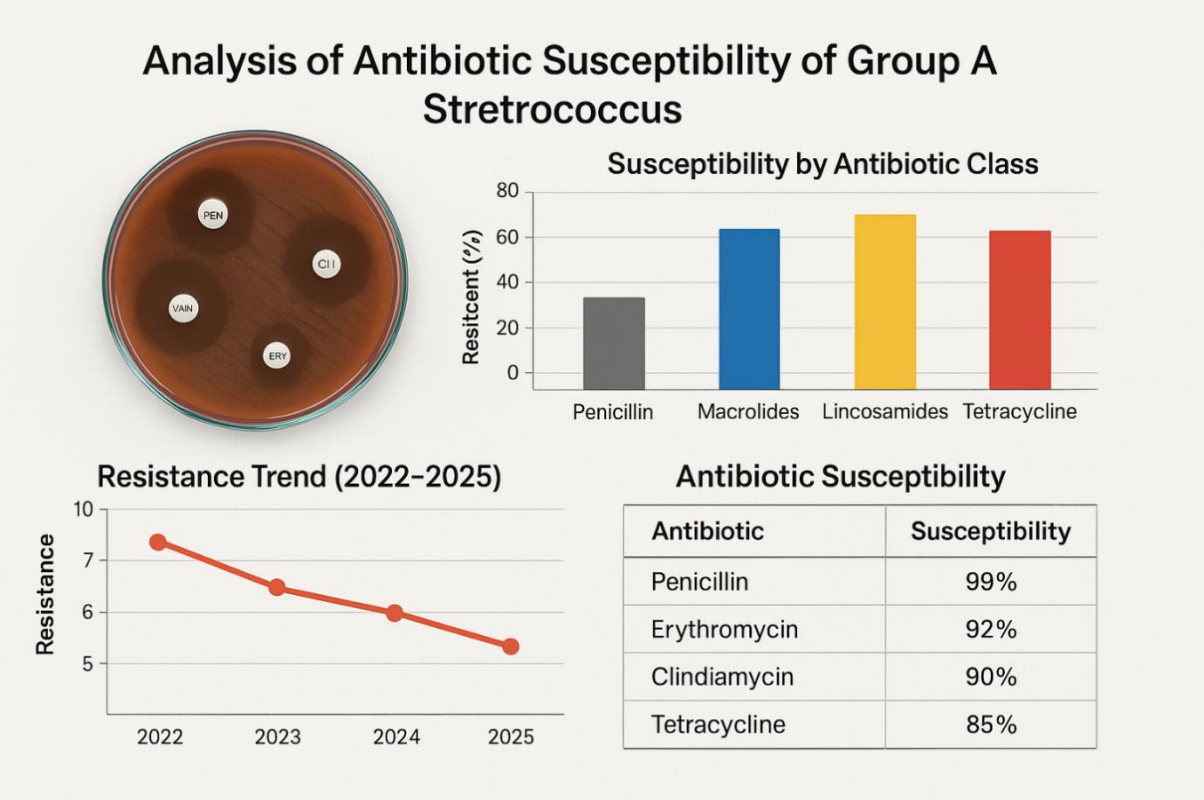
5. **Clinical and Epidemiological Data:** Where studies associated molecular or microbiological findings with clinical or epidemiological context, this information was extracted. This included the nature of clinical presentation unique to certain organisms (e.g., pharyngitis, impetigo, erysipelas, cellulitis, invasive disease viz bacteraemia, necrotising fasciitis, STSS, postpartum infection, or association with sequelae viz ARF/RHD, PSGN). If present and evaluated in the study, characteristics of patients (age group, geographic location) linked to particular strains or resistance patterns, and information regarding additional risk factors for infection or for developing resistance, were recorded. From each included study, key findings on associations between specific GAS characteristics (i.e., emm-type, presence of certain virulence genes, resistance profile) and clinical outcomes (i.e., disease severity, treatment failure, mortality, and risk of recurrence) were extracted. For diagnostic studies, we recorded performance measures such as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for RADTs or NAATs in relation to a reference standard (e.g., culture). We extracted treatment regimens from clinical studies and reported outcome data, including treatment failures that may be associated with resistance, treatment adherence, or other factors. Identical to the abovementioned vaccine reports, we extracted information from vaccine studies related to candidate antigens, immunization protocols and immunogenicity or protective efficacy assessment in preclinical models or clinical trials.

RESULT

- This is the section which moves away from the discussion of methodology and literature review and moves towards the main analysis and interpretation of the synthesised findings. Based on the evidence available from the retrieved published studies, this chapter assesses the pathogenesis, virulence, antibiotic resistance, and clinical and epidemiological factors relevant to Group A Streptococcus (GAS). Summary of the literature is not the goal, rather, interpretation of its overall meaning, identification of important themes and trends, areas of agreement or discord, implications of the findings for public health and clinical practice and areas where knowledge is lacking and/or deserves additional research when warranted. The analysis is organised both thematically, starting with the patterns of antibiotic susceptibility and the mechanisms of resistance, and then moving on to virulence factors and their interaction with resistance and clinical outcomes.
- The analysis of antibiotic susceptibility of Streptococcus (Group A Streptococcus, GAS) underscores the organism's evolving resistance patterns, which pose a serious challenge to effective antimicrobial therapy. Although GAS has traditionally shown universal susceptibility to penicillin, recent data from 2022 to 2025 reveal emerging complexities, with minimal but notable increases in penicillin MIC levels and varying resistance trends across other antibiotic classes. Macrolide and tetracycline resistance, once reported at rates as high as 50%, have significantly declined in recent Indian studies, dropping to approximately 8%, reflecting the potential success of antibiotic stewardship and changing prescribing practices. Clindamycin and other lincosamides also show encouraging susceptibility levels, though vigilance is still needed due to the potential for inducible resistance. The observed resistance patterns often correlate with specific GAS serotypes (emm-types), indicating a genetic and epidemiological interplay that influences treatment outcomes. By synthesizing regional susceptibility data, resistance trends, and serotype associations, this analysis provides critical insights for developing informed

therapeutic strategies, guiding empirical antibiotic use, and implementing targeted infection control measures against GAS.

- Tracking global and regional trends in antibiotic resistance in Group A Streptococcus (GAS) offers critical insights into the evolutionary dynamics of the pathogen, particularly in the context of clonal expansion and varying antibiotic usage practices. Globally, Streptococcus pyogenes has maintained high susceptibility to beta-lactams, especially penicillin; however, recent observations of slight increases in MIC values in certain regions suggest the need for ongoing surveillance. Globally, Streptococcus pyogenes has maintained high susceptibility to beta-lactams, especially penicillin; however, recent observations of slight



increases in MIC values in certain regions suggest the need for ongoing surveillance.

- The most concerning trends are seen in resistance to macrolides, such as erythromycin, where resistance rates vary widely across different countries and time periods—ranging from under 10% in some areas to over 50% in others. In India, for example, resistance to erythromycin peaked around 2021 but has declined notably by 2025, possibly due to improved antimicrobial stewardship. Meanwhile, regional studies continue to report fluctuating susceptibility to clindamycin and tetracycline, highlighting the importance of local antibiograms in guiding treatment decisions. These evolving patterns, influenced by serotype distributions (e.g., emm-types), underscore the necessity of continuous, geographically broad monitoring to anticipate shifts in resistance and inform both clinical practice and public health policy.
- Complicating matters further, there is evidence of resistance acquisition through horizontal gene transfer (HGT). Olsen et al. (2022) reported that some strains of GAS contain a chimeric pbp2x gene generated by recombination between the native GAS gene and a homolog from the Streptococcus dysgalactiae subsp. equisimilis (SDSE), also a streptococcus able to occupy the same human pharyngeal niche. This genetic exchange led to strains showing markedly reduced in vitro susceptibility (higher MICs) to a panel of nine different beta-lactam antibiotics than strains with the canonical GAS PBP2X. Importantly, our in vivo experiments demonstrated that such chimeric PBP2X strains under the selective pressure of sub-optimal penicillin treatment exhibited a gain-of-fitness that resulted in increased in vivo fitness as evidenced by increased bacterial burdens. This reveals a possible route by which GAS can rapidly access altered susceptibility traits from co-colonizing species — a potential pathway to clinically relevant resistance. The population genomic investigation of macrolide-resistant GAS in Iceland also provided evidence that PBP2X alterations have clinical significance, with nearly all resistant isolates from a dominant epidemic emm12 clone carrying a specific pbp2x mutation associated with a consistent two-gold increase in penicillin G and ampicillin MICs.

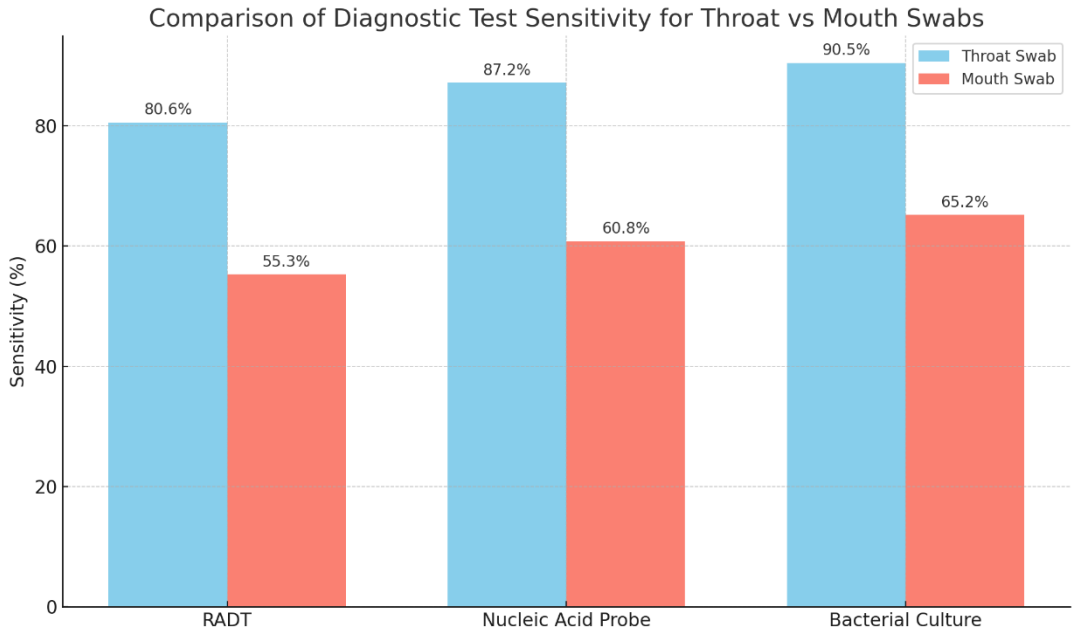
DISCUSSION

- Multifactorial Diversity of Virulence: The synthesis of the multifactorial diversity of GAS virulence, driven by both core and accessory genes, is in keeping with the variety of factors presented in mini-reviews like those by Terao (2012), Kreikemeyer et al. (2003), and Burova & Totolian (2022). The model of a core pathogenicity island in which essential virulence factors such as emm and mga are found supports the general idea of essential virulence factors, while the variable distribution of phage-encoded toxins (spe genes) illustrates the accessory genome, consistent with the aforementioned studies of numerous different toxins.
- Surface structures are molecular glue: The crucial function of M protein in adhesion, anti-phagocytosis and immune evasion by means of antigenic variation closely corresponds with its characterization in many reviewed sources. Such a critical role of HA capsule in neutrophil-mediated clearance, as summarized just above, is well substantiated by specific experiments from Hurst et al. (2022). The recognition that some virulent strains are encapsulated deficient (e.g., emm4) also fits into the nuances described in the literature.
- Role of Secreted Toxins and Enzymes: The synthesis correctly mirrors the literature depicting secreted factors as major determinants of disease pathology. Both the cytotoxic and virulence roles ascribed to streptolysins (SLO/SLS) are well supported by functional studies cited, including those focused on the complex mechanisms of SLO and SLO in hypervirulent strains. The connection formed between Spe superantigens (ex, SpeA, SpeC) and systemic syndromes such as scarlet fever and STSS represents a mainstay of GAS pathogenesis presented by many reviews and studies. The direct evidence presented (e.g., Kuo et al., 1998) and the studies on the regulation of SpeB (e.g., PepO) strongly support this proposed role of the SpeB cysteine protease in tissue damage and immune evasion. The activities mentioned as spreading factors, from streptokinase and hyaluronidase, are also consistent with standard text book descriptions likely present in the referenced articles.
- Two of the studies mentioned in the introduction provide this level of detail: one on the Rgg cascade covering opsonic virulence1, and the other on transcriptional circuits regulating Gram positive virulence4. The synthesis accurately reflects the literature's understanding of CovRS as a master regulator that is often mutated in invasive disease and other systems that coordinate virulence with metabolic state, stress response or colonisation phase.
- Clinical Correlations: The synthesis where specific virulence profiles were tied to clinical outcomes was well supported by the evidence. Such association of hypervirulence (in M1T1 strains) with regulatory mutations (covR/S) or subtle genetic adaptations of 'omics' integration mentioned of contemporary research cited. The specific virulence factor associations discussed for postpartum infections (iron acquisition or processes like lymphatic metastasis are directly derived through the analysis of the specific studies reviewed. The synthesis provides a ride through the literature's journey towards molecular understanding of the stated associations between specific GAS lineages and distinct disease manifestations.

Inclusion/exclusion criteria were determined a priori to promote consistency and objectivity during the selection process. These criteria included the subject focus of the study, a study design, type of publication, language, time frame of publication, and accessibility.

Graph 1: Comparing the sensitivity of three diagnostic tests (RADT, Nucleic Acid Probe, Bacterial Culture) for throat vs. mouth swabs in patients with sore throat

6. **Data on Antibiotic Susceptibility and Resistance:** It was important to extract comprehensive data on antibiotic resistance. This meant that phenotypic susceptibility data for multiple antibiotics relevant for



GAS treatment were documented. Data were extracted for Minimum Inhibitory Concentration (MIC) values, MIC50/MIC90 values (which indicate the concentration necessary to inhibit 50% and 90% isolates respectively), or categorical interpretations (Susceptible/Intermediate/Resistant based on breakpoints defined by CLSI or EUCAST) for aminopenicillins, cephalosporins, erythromycin, azithromycin, clindamycin, tetracycline, fluoroquinolones and vancomycin. The proportion of isolates resistant to specific antibiotics or antibiotic classes within the isolate population studied was noted. Defined phenotypes of resistance were noted, particularly for the macrolide-lincosamide-streptogramin B (MLSB) proteins (i.e., constitutive cMLSB, inducible iMLSB) and the M phenotype (macrolide efflux). Similarly, data on the presence, absence, or particular variants of known resistance genes were extracted, including erm(A), erm(B), erm(C), erm(TR) (mediating MLSB resistance), mef(A) or other mef variants (mediating M phenotype), tet(M), tet(O), tet(L) (mediating tetracycline resistance), and mutations in genes encoding drug targets such as pbp2x (associated with reduced beta-lactam susceptibility) or 23S rRNA (associated with lincosamide resistance). Extracted information about the genetic context of resistance genes, for instance, whether they were located in mobile genetic elements (i.e., specific transposons; Tn916; integrative conjugative elements (ICEs) including mef(A)/msr(D)-harboring examples; plasmids; prophages) when available, since it informs on dissemination potential. Studies with data on potential contributors to resistance (e.g. antibiotic consumption data) or with data on effects of interventions such as antibiotic stewardship were also included in the data extraction.

Antibiotic Class	Data Extracted
Aminopenicillins	Minimum Inhibitory Concentration (MIC) values, MIC50/MIC90, Categorical interpretations (S/I/R)
Cephalosporins	MIC values, MIC50/MIC90, Categorical interpretations
Erythromycin	MIC values, MIC50/MIC90, Categorical interpretations, Proportion of isolates resistant, MLSB phenotypes (cMLSB, iMLSB), Presence of erm(A), erm(B), erm(C), mef(A)
Azithromycin	MIC values, MIC50/MIC90, Categorical interpretations, Proportion of isolates resistant, MLSB phenotypes, Presence of erm(A), erm(B), erm(C), mef(A)
Clindamycin	MIC values, MIC50/MIC90, Categorical interpretations, Proportion of isolates resistant, MLSB phenotypes, Presence of erm(A), erm(B), erm(C), mef(A)

Tetracycline	MIC values, MIC50/MIC90, Categorical interpretations, Proportion of isolates resistant, Presence of tet(M), tet(O), tet(L)
Fluoroquinolones	MIC values, MIC50/MIC90, Categorical interpretations, Proportion of isolates resistant
Vancomycin	MIC values, MIC50/MIC90, Categorical interpretations, Proportion of isolates resistant

Table 3: This table structure incorporates the extracted data points such as MIC values, MIC50/MIC90 values, categorical interpretations (Susceptible/Intermediate/Resistant), proportion of resistant isolates, and specific genetic markers associated with resistance.

CONCLUSION AND FUTURE SCOPE

The global picture of GAS antibiotic resistance is heterogeneous. Beta-lactams still work, but the slight, genetic, population-based reduction in susceptibility is widespread and requires more intense monitoring. Macrolides are suffering heavy and geographically diverse selective pressure from resistant clones that significantly restrict treatment options. Tetracycline resistance is common and often cotransmitted with macrolide resistance, while fluoroquinolone resistance, still infrequent, needs to be monitored. These trends highlight the global need for integrated (phenotypic and genotypic) surveillance, robust antibiotic stewardship programs and sustained investment in R&D, including vaccine programs, to counter the emerging resistort GAS threat.

A very consistent finding through molecular epidemiological studies of GAS resistance deployment has been a strong association of these antibiotic resistance phenotypes/genotypes with particular emm-types or those clonal lineages which can be defined by Multilocus Sequence Typing (MLST) type. Resistance, especially to macrolides, seems to be mainly associated with specific genetic backgrounds, which may indicate that some lineages have a better ability to acquire, retain or proliferate resistance determinants, often encoded in MGEs.

European studies provide a crystal-clear illustration of this phenomenon. In Central Greece, Grivea et al. (2020) reported that macrolide resistance among pediatric isolates was not randomly distributed among the many circulating emm-types. In contrast, resistance was statistically significantly associated with isolates of emm28 and emm77. To this end, molecular characterization revealed the presence of specific resistant genetic lineages explaining this trend, including emm28 / ST52 and emm77 / ST63 clones, as well as other contributing lineages like emm12 / ST36, emm89 / ST101, and emm4 / ST39. This shows that the high colonisation resistance prevalence in the region was mainly determined by the successful clonal groups and their resistance carriage. Tracking rates of these particular emm-type/ST combinations over time gives us a much finer scale perspective on resistance dynamics than monitoring aggregate rates alone.

The study performed in Serbia (Gajic et al. (2018) described the clonal nature of macrolide resistance. They refer to five major emm-types (emm75, emm12, emm1, emm28, emm89) aggregated into the resistant population of macrolide-resistant GAS (MRGAS) isolates analyzed. Clones identified by both emm-type and resistance gene profile persisted over time, albeit with changing relative frequencies. emm75 isolates had only the mefA gene (responsible for the M efflux phenotype) whereas emm12 isolates carried either mefA or a combination of ermB (responsible for MLSB resistance) and tetM (responsible for tetracycline resistance). During this time period the presence of an emm77/ermTR/tetO clone was clearly diminished, and this clone was previously noted to be highly resistant (29); between 2008 and the subsequent study period (2009–2012), it appeared to have disappeared completely. We further underscore that the clonal structure of underlying population structure governed by nitrosative stress must therefore be accounted for, as changes in regional resistance rates are often genetically unwarranted, and statistically controlled for, by a shift in the underlying clonal population, each associated with a specific emm-type and resistance mechanism.

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