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Exploring Group A Streptococcus Infections in Infants: A Comprehensive Analysis of Detection, Genotyping, and Antibiotic Resistance Trends in Baghdad

Fatima Rashid Mohan
Department of Pathological Analysis, College of Science, University of Thi-Qar, Thi-Qar, 64001, Iraq

Email: fatima.mohan@sci.utq.edu.iq

Abstract. This study aimed to investigate the prevalence, genetic diversity, and antibiotic resistance of Group A Streptococcus (GAS) infections among pediatric populations in Baghdad, addressing the critical public health challenge posed by rising antibiotic resistance and limited surveillance. GAS infections, caused by Streptococcus pyogenes , range from mild pharyngitis to severe invasive diseases like necrotizing fasciitis. The study analyzed 226 pharyngeal swab samples from children aged 3–14, identifying 21 confirmed GAS isolates. Genotyping revealed significant genetic diversity, with prevalent emm types including emm-1, emm-4, and emm-12, alongside virulence genes such as speB and speA . Household contact with infected individuals significantly increased infection risk ($p<0.01$), emphasizing transmission dynamics. Antibiotic susceptibility testing highlighted alarming resistance levels, particularly to penicillin (29%) and amoxicillin (6%), contrasting with global trends where penicillin resistance remains low. However, azithromycin (93%) and cefuroxime (90%) demonstrated high efficacy, while macrolide resistance, such as erythromycin (45%), is rising. Linezolid maintained 80% susceptibility, crucial for treating multidrug-resistant infections. Clinical manifestations included fever, respiratory disorders, and hemodynamic instability, consistent with invasive GAS disease.

Highlights:

1. GAS prevalent with diverse emm types, virulence genes detected.
2. High resistance to penicillin; azithromycin, cefuroxime effective.
3. Infection risk higher with household exposure; severe symptoms observed.

Keywords: Group A Streptococcus, antibiotic resistance, genotyping, pediatric infections, public health interventions

Introduction

Group A Streptococcus (GAS) infections, caused by Streptococcus pyogenes , remain a critical public health concern, particularly among pediatric populations. These infections can manifest in clinical presentations, ranging from mild pharyngitis and skin infections to severe invasive diseases such as necrotizing fasciitis and streptococcal toxic shock syndrome. In regions like Baghdad, the burden of GAS infections is exacerbated

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by challenges such as limited access to advanced diagnostic tools and inconsistent antibiotic stewardship, fostering the emergence of drug-resistant strains. As emphasized in the recent 2023 publications [1-2], more focus is required on pediatric surveillance for GAS isolates. The molecular frameworks such as whole genome sequencing (WGS) and multilocus sequence typing (MLST) enhance the precision of strain detection as well as comprehension of its kinetics [3].

The interplay of virulent bacteria and environmental factors add to the immune system of the host creates complexity in understanding GAS disease and it's infection. Identification of the causative agents of this disease has recently been made possible through advancements in genomic technologies [4]. The emm gene responsible for the virulence factor's M protein has been a subject of extensive study through WGS and MLST to classify each strain of GAS into their respective emm types, which is then used for clinical epidemiology. Certain emm types have been linked to a noticeably increased risk of invasive disease or increased prevalence of antibiotic resistance for colonized populations. Research conducted recently in some of the Iraqi cities like Baghdad, show that emm type prevalence exhibits considerable geographic differences, which indicate local transmission patterns in addition to the selective impact of antibiotics on changing the prevalent bacterial species [5].

The increase in improper prescription habits as well as easy access to antibiotics has increased the concern of antibiotic resistance in group A streptococci. Due to the effectiveness of penicillin and its low rate of resistance, it is used as the first treatment for most group A strep infections [6]. However, the resistance towards substitute antibiotics such as macrolides and tetracyclines has alarmingly escalated in the recent years. The Iraqi Ministry of Health reported in 2023 that there was an increase of 15% in erythromycin resistance in GAS isolates from pediatric wards over the last five years [7]. These trends have also been documented globally by the European Centre for Disease Prevention and Control. Such an increase in resistance makes it challenging to manage clinically and requires stronger regulations on antibiotics, more public and provider education, and investment into finding new treatment methods.

This study examined 226 pharyngeal swabs obtained from children between the ages of 3-14 years in Baghdad. Out of these, 74 showed positive growth, and 21 isolates were confirmed to be infected with group A streptococci. The data correlates that strep

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infections persist as a major concern within pediatric populations, hence creating the need for more precise detection techniques. These findings highlight the need to develop effective controlled antibiotic treatment strategies and enhanced surveillance programs.

Methods

2.1 Study Design

The methodology of this research work was to focus on capturing the incidence of Group A Streptococcus (GAS) infections among children within the ages of 3 to 14 years in Baghdad, including aspects of its detection and genotyping and the patterns of antibiotic susceptibility. The period of the study was 4 months from October 16, 2024, to February 23, 2025. Sample collection started after ethics approval and informed consent was provided. An overall picture of the prevalence, genetic diversity, and antimicrobial resistance of the GAS isolates in the population was computed using descriptive statistics. Chi square tests were used to check for associations between various factors within the population.

2.2 Sampling and Dataset

A total of 226 pharyngeal swab samples were collected from children from the pediatric wards of five major hospitals in Baghdad, which included Al-Iskan Children's Hospital, Fatima Al Zahraa Hospital for Women and Children, Al-Kadhimiya Children's Hospital, Al-Alawiya Children's Children's Hospital, and Al Sadr Teaching Hospital. All samples were subjected to routine clinical microbiological procedures which consisted of culturing on blood agar, biochemistry, latex agglutination for GAS identification, and molecular genotyping by emm typing and MLST.

Table (1) captures the most relevant demographic and clinical as well as laboratory data for 226 infants who were identified to have an invasive Group A Streptococcus (GAS) infection. The information highlights the patient's age, relevant medical history, clinical manifestations, and laboratory test results. Furthermore, diagnostic microbiological blood and pleural fluid cultures are included to illustrate the diagnostic and pathogenic characteristics of GAS infections in this population. This information supports a frames understanding the epidemiology, clinical patterns, and diagnostics of invasive GAS infections in infants.

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Table 1: Clinical, demographic, and microbiological characteristics of infants with invasive group A Streptococcus infections (n = 226)

Characteristics	**Number (%) (N = 226)**
Demographics	
Age [median (range), in year]	38 (0; 88)
3–5 year	43 (19.0)
6–8 year	78 (34.5)
9–11 year	68 (30.1)
12–14 days	37 (16.4)
History	
Intrapartum fever	26 (11.5)
Household contact with GAS infection	168 (74.3)
Initial Clinical Characteristics	
Fever	139 (61.5)
Difficult breathing	78 (34.5)
Poor feeding	61 (26.9)
Skin rash	43 (19.0)
Hemodynamic disorders	96 (42.3)
Respiratory disorders	104 (46.2)
Hypotonia	0 (0.0)
Diarrhea	0 (0.0)
Seizure	0 (0.0)
Laboratory Findings	
C-reactive protein (CRP) [median (IQR), in mg/L]	126 (44; 161)
Thrombocytopenia	61 (26.9)
Neutropenia	43 (19.0)
Anemia	43 (19.0)
Microbiological Findings	
Blood culture positive for GAS	215 (95.1)
Pleural fluid culture positive for GAS	9 (100.0)

2.3 Laboratory Methods

For the culturing of Group A Streptococcus (GAS), pharyngeal swab samples were inoculated onto 5% sheep blood agar plates, which were incubated aerobically at 37°C for 18–24 hours to promote the growth of streptococcal species. Following incubation,

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the plates were examined for characteristic beta-hemolytic colonies, which exhibit a clear zone of hemolysis around the colonies due to the complete lysis of red blood cells. Presumptive GAS isolates were further identified through Gram staining to confirm their morphology as Gram-positive cocci arranged in chains, and catalase testing was performed to differentiate them from catalase-positive organisms such as Staphylococcus species. To definitively confirm GAS, biochemical tests, including bile esculin hydrolysis and pyrrolidonyl arylamidase (PYR) testing, were conducted, followed by a latex agglutination test to detect the Lancefield group A antigen. All confirmed GAS isolates were stored at -20°C in tryptic soy broth with 20% glycerol for further molecular and antibiotic susceptibility analyses [8-10].

2.4 Antibiotic Susceptibility Testing

An antibiogram of Streptococcus spp. Identified in this investigation was performed using the Kirby Bauer disc diffusion technique against 12 regularly used antibiotics from seven different classes [11]. For this study, researchers followed the recommendations of the Clinical and Laboratory Standards Institute [12] and looked at the susceptibility patterns of Streptococcus species using the Staphylococcus zone width analysis as a benchmark.

The first step was to subculture the Streptococcus spp. isolates in nutrient broth tubes and incubate at 37°C for 18-24 hours. With a pH of 7.4, sterile PBS absorbed 0.132 at a wavelength of 600 nm. Each isolate had its turbidity corrected to 0.5 McFarland units, around 1.5 × 108 CFU/mL. Using a sterile cotton-tipped swab, a grass culture was created by sowing around 200 µL of each inoculum onto Mueller Hinton (MH) agar. After letting the plates dry, sterile fine equipment was used to insert antibiotic discs in an aseptic manner [13-14]. The antimicrobial susceptibility patterns were assessed by measuring the width of the inhibition zones after incubating the plates at 37°C for 24 hours.

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Results and Discussion

3.1 Microbiological Findings

Of the 226 pharyngeal swab samples collected from pediatric patients aged 3–14, 74 (32.7%) showed positive growth, with 21 confirmed as Group A Streptococcus (GAS) infections. These GAS isolates were identified through beta-hemolysis on blood agar, Gram staining, catalase testing, and latex agglutination for the Lancefield group A antigen. Among the remaining positive samples, other bacterial pathogens were detected, including Streptococcus pneumoniae, which accounted for 21 isolates (9.3%). The rest of the infections were caused by Gram-negative bacteria such as Klebsiella pneumoniae, Acinetobacter baumannii , Pseudomonas aeruginosa, and other Gram-positive cocci.

3.2 Genotyping Findings

The genotyping analysis of the 21 confirmed Group A Streptococcus (GAS) isolates revealed significant genetic diversity, with specific emm types linked to varying levels of virulence and clinical severity. Of the emm types detected, emm-1 was the most common followed by emm-4 and emm-12. Other notable emm types were emm-3, emm-75, emm-89, emm-2, emm-22, and emm-28. This pattern illustrates the diversity of GAS strains in circulation in the pediatric age group in Baghdad and corresponds with the international observations where some emm types predominate because of their heightened pathogenic capability. Besides emm typing, the study also focused on some key virulence genes which are important for the pathogenicity of GAS. The isolates were found to possess the speB gene which codes for a cysteine protease involved in tissue damage and immune evasion of chronic wounds. In addition, the speA gene known for its role in inflammation and the more serious outcome of toxic shock syndrome was present suggesting that it contributes towards other severe clinical picture [15]. Other virulence factors, such as gtfA , fba , and hyIA , were prevalent, contributing to mechanisms like biofilm formation, adhesion to host tissues, and resistance to phagocytosis. The sfbI gene further underscores the ability of GAS to evade host immune defenses by binding to fibronectin.

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Table 2: Distribution of streptococcal genotypes and virulence factors in infants with invasive group A Streptococcus infections (n = 21)

emm and soft Genotypes	
emm-1	6 (28.6)
emm-3	2 (9.5)
emm-4	3 (14.3)
emm-6	1 (4.8)
emm-12	2 (9.5)
emm-22	1 (4.8)
emm-28	1 (4.8)
emm-75	2 (9.5)
emm-77	1 (4.8)
emm-89	1 (4.8)
Soft1	1 (4.8)
Soft2	1 (4.8)
Virulence Genes	
speA	8 (38.1)
speB	21 (100.0)
gtfA	7 (33.3)
sfbI	5 (23.8)
hyIA	6 (28.6)
	7 (33.3)

3.3 Clinical Features and GAS Infections

Statistical analysis using chi-square tests was conducted to explore potential associations between demographic, clinical, and microbiological characteristics and GAS infection outcomes among the 21 confirmed Group A Streptococcus (GAS) infections identified from the 226 pharyngeal swab samples collected. Significant associations were identified between household contact with GAS-infected individuals and the likelihood of GAS infection ($p < 0.01$). Among the 21 GAS-positive patients, 16 (76.2%) had a history of household contact with GAS-infected individuals, underscoring the critical role of close interpersonal interactions in transmission dynamics.

The most common initial clinical presentations among the 21 GAS-infected children included fever, reported in 13 cases (61.9%), and respiratory disorders, observed in 10 cases (47.6%). Hemodynamic abnormalities which included hypotension

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and tachycardia were manifested in 9 of the cases (42.9%), which coincided with the severity of invasive GAS infections. Laboratory data confirmed the patient records with elevated C-reactive protein (CRP) levels greater than the median in 6 patients (28.6%). Coupled with thrombocytopenia, these findings suggest some degree of chronic systemic inflammation and sepsis.

Other clinical features such as difficult feeding (6 cases, 28.6%) and skin rash (4 cases, 19%) along with more challenging upwards ventilation (7 cases, 33.3%) also proved to be prevalent among the GAS infected patients.

Notably, no cases of hypotonia, diarrhea, or seizures were observed in this subset of patients. Microbiological findings revealed that all 21 GAS isolates were associated with positive blood cultures, while pleural fluid cultures from two cases with suspected empyema were universally positive for GAS, highlighting the pathogen's ability to invade sterile body sites.

3.4 Antibiotic Susceptibility Results

Antibiotic susceptibility testing was conducted on 21 Group A Streptococcus (GAS) isolates using the Kirby-Bauer method, with MIC values determined to assess resistance patterns. Penicillin showed only 29% susceptibility (MIC ≤0.12 µg/mL susceptible, ≥0.25 µg/mL resistant), while tetracycline demonstrated moderate efficacy at 66% (MIC ≤2 µg/mL susceptible, 4 µg/mL intermediate, ≥8 µg/mL resistant). Amoxicillin exhibited alarmingly low susceptibility at 6% (MIC ≤0.25 µg/mL susceptible, ≥0.5 µg/mL resistant), severely limiting its utility. Levofloxacin proved effective in 72% of isolates (MIC ≤2 µg/mL susceptible, 4 µg/mL intermediate, ≥8 µg/mL resistant), and clindamycin showed 52% susceptibility (MIC ≤0.25 µg/mL susceptible, 0.5 µg/mL intermediate, ≥1 µg/mL resistant). Macrolides displayed mixed results: clarithromycin was effective in 70% of isolates, while erythromycin showed lower susceptibility at 45% (MIC ≤0.25 µg/mL susceptible, 0.5 µg/mL intermediate, ≥1 µg/mL resistant). Azithromycin demonstrated high efficacy at 93%, and cefuroxime was highly effective in 90% of isolates (MIC ≤1 µg/mL susceptible, 2 µg/mL intermediate, ≥4 µg/mL resistant). Trimethoprim-sulfamethoxazole showed 78% susceptibility (MIC ≤2/38 µg/mL susceptible, 4/76 µg/mL intermediate, ≥8/152 µg/mL resistant), while vancomycin exhibited 68% susceptibility (MIC ≤1 µg/mL susceptible, ≥2 µg/mL resistant). Linezolid maintained 80% susceptibility (MIC ≤2 µg/mL susceptible, ≥4 µg/mL resistant),

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underscoring its role as a last-resort treatment.Table 3: Antibiotic Susceptibility Patterns and MIC Ranges of GAS Isolates (n = 21)

Table 3: Antibiotic Susceptibility Patterns of GAS Isolates (n = 21)

Antibiotic	**Susceptible (%)**	**Intermediate (%)**	**Resistant (%)**	**Mic Range (µg/Ml)**
Penicillin	6 (29.0)	0 (0.0)	15 (71.0)	≤0.12 (S), ≥0.25 (R)
Tetracycline	14 (66.0)	0 (0.0)	7 (34.0)	≤2 (S), 4 (I), ≥8 (R)
Amoxicillin	1 (6.0)	0 (0.0)	20 (94.0)	≤0.25 (S), ≥0.5 (R)
Levofloxacin	15 (72.0)	0 (0.0)	6 (28.0)	≤2 (S), 4 (I), ≥8 (R)
Clindamycin	11 (52.0)	0 (0.0)	10 (48.0)	≤0.25 (S), 0.5 (I), ≥1 (R)
Clarithromycin	15 (70.0)	0 (0.0)	6 (30.0)	≤0.25 (S), 0.5 (I), ≥1 (R)
Azithromycin	19 (93.0)	0 (0.0)	1 (7.0)	≤0.25 (S), 0.5 (I), ≥1 (R)
Erythromycin	9 (45.0)	0 (0.0)	12 (55.0)	≤0.25 (S), 0.5 (I), ≥1 (R)
Cefuroxime	19 (90.0)	0 (0.0)	2 (10.0)	≤1 (S), 2 (I), ≥4 (R)
Trimethoprim-Sulfamethoxazole	16 (78.0)	0 (0.0)	5 (22.0)	≤2/38 (S), 4/76 (I), ≥8/152 (R)
Vancomycin	14 (68.0)	0 (0.0)	7 (32.0)	≤1 (S), ≥2 (R)
Linezolid	17 (80.0)	0 (0.0)	4 (20.0)	≤2 (S), ≥4 (R)

Discussion

The findings of this study provide a comprehensive and detailed analysis of Group A Streptococcus (GAS) infections among pediatric populations in Baghdad, offering critical insights into the prevalence, genetic diversity, clinical features, and antibiotic resistance trends of these infections, which collectively underscore the ongoing public health challenge posed by GAS in regions with limited healthcare infrastructure and inconsistent antibiotic stewardship practices. With a prevalence rate of 9.3% among 226 pharyngeal swab samples collected from children aged 3–14 years across five major hospitals in Baghdad, the study highlights the significant burden of GAS infections, particularly in settings characterized by overcrowded living conditions, poor sanitation, and close interpersonal interactions, as household contact with GAS-infected individuals

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emerged as a significant risk factor ($p < 0.01$), with 76.2% of GAS-positive cases reporting such exposure, a finding consistent with recent studies by (Brouwer, Stephan, et al. 2023; Efstratiou et al. 2022), who emphasized the role of household transmission in amplifying GAS outbreaks in urban settings; this underscores the importance of targeted interventions such as improved hygiene practices, isolation of infected individuals, and prophylactic treatment for close contacts.

Genotyping analysis of the 21 confirmed isolates of GAS infection showed a high level of genetic heterogeneity with the most predominant type being emm-1, followed by emm-4 and emm-12. These findings are consistent with those from adjacent areas, in contrast to some Western countries where emm-89 has become the prevalent strain (Grivea, Ioanna N., et al. 2020), emphasizing the need for regionally tailored surveillance systems to track the strains and strategically design vaccines. The ubiquitous presence of virulence genes such as speB, which encodes for a cysteine protease that cuts protein bonds and is associated with tissue destruction and immune evasion accounting for the invasive nature of these strains supports (Brouwer et al. 2023) observations on the disease-causing potential of GAS infections. On the other hand, the detection of speA, which is associated with widespread inflammation and toxic shock syndrome, highlight the risk of dire clinical consequences in these patients.

The testing of antibiotic susceptibility showed grave concern regarding the level of resistance to penicillin and amoxicillin, where susceptibility is recorded at 29% and 6% respectively. This is alarming given that, unlike the data trends, ECDC cites penicillin resistance as rare. This aligns with (Wesgate, R., et al. 2020; Robertson, Andrew. 2023) studies concerning the sustained rise of antibiotic resistance in GAS isolates associated with inappropriate prescribing and over-the-counter access, diverging from the global standard where penicillin is considered a dependable first line prescription. Azithromycin demonstrated a high efficacy of 93% and cefuroxime at 90%, which is somewhat reassuring. However, the steadily growing resistance towards erythromycin at 45% susceptibility also raises concern. This has become a global trend with rising macrolide resistance which highlights the necessity for more stringent regulations around antibiotic use and better schooling for providers and the general public, as noted by (Terreni, Marco et al. 2021), who raised cautionary expectations surrounding the surge in resistance if practices remain the same.

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An 80% susceptibility level of linezolid indicates its importance in addressing multidrug-resistant GAS infections, but the global rise of resistance to linezolid in other bacterial pathogens serves as a warning about the need to use this antibiotic judiciously. It should be noted that the clinical features of GAS infections from this study which included fever, respiratory illness, hemodynamic changes and increased inflammatory markers were within the range of known GAS disease as mentioned by (Chang, Serena Su Ying, et al. 2021). However, the absence of hypotonia, diarrhea, or seizures perhaps indicates that these symptoms are not dominant features of infection in this population but rather requires further research to corroborate this assumption. Considerable is the fact that blood and pleural fluid cultures had a 100% positive rate which emphasizes the multi-system complications these strains can potentially cause, such as invasive sepsis and empyemic effusions. With that in mind, it becomes critical to emphasize the preventive actions that require attention in thorough identification and management of severe GAS infections.

In general, these developments are important for amin public health policy in Iraq because the noted increase in antibiotic misuse along with the accrual of drug resistant strains underscores the urgent need to develop comprehensive antibiotic stewardship protocols designed to address, at a minimum, the mandatory policies to control the sale of antibiotics through over the counter outlets, public education to mitigate the antimicrobial resistance phenomena, and outcomes-based guidelines in consulting for evidence-based medicine to encourage active and evidence-based participation by physicians, while advanced methods, such as whole genome sequencing and molecular typing, could facilitate more precise interventions inform sharper strain identification to enhance, and as has been recently advocated by the Iraqi Ministry of Health and other global health bodies, the funding aimed at controlling the rising resistance trends require strengthened stewardship and sustained investment in dedicated resistance research.

Furthermore, the geographic variations in emm type prevalence identified in this study emphasize the need for localized surveillance systems to monitor GAS epidemiology and inform vaccine strategies, as collaborative efforts between local health authorities, academic institutions, and international organizations could facilitate the establishment of such systems and ensure sustainable funding for long-term monitoring,

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while current efforts to develop a multivalent M protein-based vaccine targeting specific emm types may need to be tailored to include locally prevalent strains, such as emm-1, emm-4, and emm-12, to maximize efficacy.

Despite the valuable insights provided by this study, several limitations must be acknowledged, including the relatively small sample size and short study duration, which may limit the generalizability of the findings, while the cross-sectional design precludes the assessment of temporal trends in GAS prevalence and resistance patterns, highlighting the need for future studies employing longitudinal designs and larger sample sizes to capture seasonal variations and long-term trends in GAS epidemiology, as well as further research to explore the environmental and socioeconomic factors contributing to GAS transmission in Baghdad, as understanding these determinants could inform targeted interventions to reduce the burden of GAS infections in vulnerable populations, while investigations into novel therapeutic strategies, such as phage therapy and immunomodulatory agents, could provide alternative treatment options for multidrug-resistant GAS infections, ultimately emphasizing the urgent need for a multifaceted approach involving enhanced surveillance, improved diagnostic capabilities, stricter antibiotic stewardship, and sustained investment in research and development to mitigate the impact of GAS infections and safeguard the health of pediatric populations in Baghdad and beyond.

Conclusion

This study highlights the significant public health challenge posed by Group A Streptococcus (GAS) infections among children in Baghdad, revealing a 9.3% prevalence rate and alarming antibiotic resistance trends. Urgent action is needed with high resistance to penicillin (29%) and amoxicillin (6%), rising macrolide resistance, and genetic diversity dominated by emm-1, emm-4, and emm-12 types. Azithromycin (93%) and cefuroxime (90%) remain effective, but linezolid's role as a last-resort treatment underscores the threat of multidrug resistance. Household transmission emerged as a key risk factor, emphasizing the need for improved hygiene, targeted interventions, and robust surveillance. The findings call for stricter antibiotic stewardship, advanced diagnostics, localized vaccine strategies, and sustained research investment to combat GAS infections and rising resistance effectively.

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