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Molecular Epidemiology and Superantigen Gene Profiles of Streptococcus pyogenes from Pediatric Pharyngitis in Baghdad City

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***Abstract*— Streptococcus pyogenes (Group A Streptococcus, GAS) constitutes one of the main global causative agents of paediatric pharyngitis. However, the molecular aspects of the circulating strain in Iraq remains unknown. This study aims to determine the patterns of genetic divergence, the content of the superantigen gene, and the clonal relationships of S. pyogenes pharyngeal isolates obtained from children in Baghdad City. A total of 174 isolates were collected from three hospitals. Confirmed identifications were performed using standard medical microbiology methods. Twelve virulence gene factors were identified using PCR; emm typing was performed using 5' end hypervariable region sequencing; and clonal analysis was performed using PFGE of SmaI-digested DNA and the resulting dendrograms. Among the 21 recognised emm types, the most frequent were emm12, emm22, and emm1. Core virulence genes (scpA, sagA, slo, and nga) were evenly distributed among the isolates, whereas retention of the superantigen genes was discordant. This work is novel in that it examines the molecular profile of S. pyogenes in Baghdad City and situates its diversity within a global and regional clonal framework, along with its associated virulence traits.**

***Keywords*— Group A Streptococcus, pharyngitis, emm typing, superantigen genes, molecular epidemiology.**

I. INTRODUCTION

The health problems caused by Streptococcus pyogenes vary in severity. Some of them can cause, pharyngitis and impetigo, are mild, while others, such as toxic shock syndrome and necrotising fasciitis which are life-threatening [1-2]. GAS pharyngitis is highly prevalent and affects children aged 5–15, and GAS is one of the top reasons why healthcare encounters lead to the prescription of antibiotics [3]. Lower- and middle-income countries face GAS and the significant health problems could occur. GAPS continue to struggle with public health systems due to inadequate health care and insufficient, affordable, and accessible services [6,7]. GAS pharyngitis can cause ARF and rheumatic heart disease, which are severe but rare health problems [4-5]. ARF and rheumatic heart disease are highly prevalent in the Middle East, parts of Africa, and some regions in South Asia [6-7].

The pathogenicity of this organism relies on individuals and aggregate immune evasion, tissue colonisation, and host damage caused by virulence factors [8]. One such virulence factor is the M protein. The M protein is a surface protein that has a dilute filamentous pattern encoded by the emm gene; the hypervariable N-terminal region of the M protein is the region of protective antibodies, and determining the more than 250 emm types is a more important predictor for the pathogenicity of GAS than any other region [9-10]. GAS also possesses other pathogenicity-increasing factors, such as superantigens and exotoxins, including SpeA, SpeC, SSA, and others, that enhance streptococcal pathogenicity by inducing a severe, systemic cytokine storm and T-lymphocyte non-specific activation [11-12]. GAS also possesses a range of other, more conserved and ubiquitous factors, the synthesis of which contributes to the strains' ability to evade the complement system and phagocytosis, survive intracellularly, and infect new hosts. Therefore, molecular characterisation of these strains with pathogenic virulence genes from various strains will enable understanding of the dynamics of transmission and the potential for virulent, invasive capability [13-14].

The last several years in molecular epidemiology have been marked by emm typing, which is considered an international gold standard due to its robustness, discrimination, and integration with CDC databases [15]. Recent papers [16-18] have confirmed once and for all that the most common types, along with a few others, are responsible for several pharyngeal and/or invasive cases worldwide: emm1, emm12, emm28, emm89, and emm4. That said, there are significant geographical differences. Specific types have been observed to sporadically or epidemically spread in certain areas; for instance, the Eastern Mediterranean region has reported several cases of emm22 pharyngiti [19-20]. while some parts [21] have noted an unprecedented increase in the types emm75 and emm87.

Along with emm typing, pulsed-field gel electrophoresis (PFGE) remains one of the most discriminative techniques for establishing the clonal relationships and tracking strain outbreaks [22]. Although PFGE has primarily been replaced

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by whole-genome sequencing (WGS) in affluent regions, it remains highly useful in resource-limited settings, particularly for associating specific PFGE clusters with virulence and clinical outcomes [23]. The combined analyses of emm type, SAg genotype, and PFGE pattern have provided remarkable lineage-specific associations, an almost complete prevalence of speA by emm1 strains, and the consistent presence of speB, slo, and nga across several otherwise distinct lineages, thus illustrating the co-evolution of virulence and clonality [24].

The global GAS surveillance networks have significant gaps, the most critical being in Iraq, which has a documented high burden of paediatric respiratory infections and surging acute rheumatic fever (ARF) reports [25], yet suffers from the absence of molecular data. Existing research from the country focuses primarily on the phenotypic characterisation of GAS and provides little to no analysis of circulating emm types, superantigen repertoires, and clonal structure. The absence of this molecular data has hindered the implementation of critical public health interventions, and, in the case of the M-protein vaccines, which are recently developed and entering the most advanced stages, HIF is aimed at the emms, which are the most commonly encountered worldwide.

This study is the most advanced to date in the molecular epidemiology of GAS pharyngitis in Iraq and will assist future surveillance, vaccine development, and improvements in GAS management. It is the molecular epidemiology of Iraqi GAS pharyngitis, which has documented and differentiated virulence factors to improve the management of GAS, including the cloning of GAS strains.

II. Materials and Methods

A. Study Design and Setting

The purpose of this cross-sectional study was to investigate the genetic diversity, virulence factors, and clonal relatedness of S. pyogenes isolates recovered from children with a complaint of sore throat in Baghdad City. This study was conducted between March 3, 2020, and August 18, 2025, with a total of one hundred seventy-four clinical isolates (n=50) obtained from Yarmouk Teaching Hospital, Medical City Hospital (n=76), and Al-Kindi Teaching Hospital (n=48). These hospitals treat many paediatric cases in Baghdad City and its surrounding governorates.

In summary, although sample are not representative of all Baghdad healthcare facilities, clinically this reflects a snapshot of circulating S. pyogenes and permits a detailed analysis of the distribution of emm types, essential/superantigenic virulence genes' carriage and clone relationships by PFGE

B. Study Population and Sample Collection

The dataset used for this sample had pertinent clinical data and throat swabs from eligible study participants. Samples comprised children aged 3-15 years with clinical complaints of pharyngitis. Eligible children had clinical complaints of a sore throat and/or fever and any of the following symptoms: exudative tonsillitis and/or cervical lymphadenopathy. Children with a history of receiving an antibiotic prescription within the prior week were excluded to reduce the potential for culture suppression. Trained hospital staff collected throat swabs using pre-sterilised rayon applicator sticks and, when applicable, skipped the tonsillar pillars and posterior pharyngeal wall. Each swab was placed in Amies transport medium and transported by hand to the microbiology lab for processing within 1–2 hours of sample collection. To avoid duplicate records per patient, only 174 unique S. pyogenes clinical isolates were obtained from the biobanks for molecular studies.

C. Isolation and Identification of GAS

On 5% sheep blood agar, 5% CO_2 was incorporated into the incubator. Slant target culture and 24 24-hour anaerobically incubated cultures were used; the target colony, β-haemolytic Gram-positive cocci in chains, and catalase-negative were isolated and collected as additional presumptive colonies of GAS. Isolated S. pyogenes was subcultured for purity; the colonies were stored at -80 °C in tryptic soy broth with 20% glycerol until genomic DNA was analysed and S. pyogenes was confirmed at the molecular level. S. pyogenes was also confirmed in the laboratory by bacitracin disc (0.04 U) inhibition and the pyrrolidonyl arylamidase (PYR) tests, and in association with bacitracin, also routinely used in the laboratory for GAS and the other β-haemolytic streptococci.

D. Genomic DNA Extraction

Genomic DNA was extracted from GAS isolates using a standardised enzymatic lysis method optimised for Gram-positive bacteria. Briefly, overnight cultures were prepared and grown in TE buffer, followed by cell wall digestion with clay lysin (10 mg/mL) and mutanolysin, and subsequent protein digestion with protease K and sodium dodecyl sulphate (SDS). After lysis, DNA and RNA were separated, and DNA was precipitated with cold absolute ethanol, followed by phenol-chloroform extraction. Afterward, the pellet was washed and redissolved in water after air-drying. Then, the samples were subjected to Nanodrop spectrophotometry to measure concentration and purity, and their integrity was evaluated by electrophoresis on a 1% agarose gel. The samples were stored at 20 degrees Celsius until PCR amplification and molecular typing.

E. The Detection of Superantigen (SAg) Genes by PCR

Duplication of SAg and virulence factors associated with the genes emm, speA, speB, speC, scpA, sagA, slo, spyCEP, nga, sic, cpa/ceuE, and fbp54 were examined using conventional PCR. From the literature, S. pyogenes-specific primers were selected, and reactions were optimised. Standard PCR was performed in 25 μL reaction volumes, with 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 μM of each forward and reverse primer, 1 U of Taq, and 50–100 ng of template DNA. DNA amplification was performed using a thermocycler. Initial denaturation

was set at 95°C for 5 minutes, followed by 30–35 cycles of denaturation at 95°C for 30 seconds, gene-specific annealing at 50–58°C for 30 seconds, and extension at 72°C for 1 minute. The final extension was set to 72 °C for 7 minutes. PCR products were separated by electrophoresis on a 1.5-2% agarose gel containing ethidium bromide or SYBR Safe for UV detection. For each gene target, a positive control and a negative control were included to examine the reliability and specificity of the amplification.

F. Emm Gene Amplification and Typing

The emm gene encodes the M protein. Gene amplification was performed using the primers indicated by the CDC. The 5' hypervariable region of the gene was our key area of interest. Twenty-five microlitre PCR reaction mixtures were used. Standard buffer, $MgCl_2$, dNTPs, primers, and Taq polymerase were added along with 50-100 ng of genomic DNA and subjected to routine cycling. The PCR products were analysed by unsubstituted agarose gel electrophoresis to verify the presence of DNA. Cleavage was performed, and the reaction products were sequenced in both directions. After aligning and validating the sequences, we added the emm-typing sequences to the CDC database. Only sequences were used to complete the final emm type that were at least 95% similar to the reference alleles.

G. Pulse- Field Gel Electrophoresis (PFGE)

To assess the genetic relationships among the bacterial isolates, the PFGE protocol was modified from the CDC PulseNet protocol. The bacterial cells were lysed, and their contents were entrapped within agarose blocks containing lysozyme, mutanolysin, and proteinase K. The agarose blocks containing the DNA were digested with SmaI for four hours at 25 °C. PFGE systems utilising the CHEF-DRIII ran at 6 V/cm with a 25°C angle for 20–22 hours, with a 120° rotation. The starting and ending switch times were 5 and 40 seconds, respectively. Ethidium bromide was added to visualise the DNA banding after PFGE, and the banding patterns were captured and digitised with a GelDoc XR system for comparison with BioNumerics/GelCompar II software. The relationship between PFGE banding patterns was quantified using Dice coefficients, and UPGMA-generated dendrograms were produced with an 80% similarity cutoff.

H. Statistical Analysis

Some associations, particularly those involving PFGE clusters, emm types, and superantigen gene profiles, were analysed using SPSS version 26 and GraphPad Prism 9. Chi-square and Fisher's exact tests were used to analyse categorical variables; the Dice coefficient was used to assess the similarity of the PFGE banding pattern matrices.
Also, molecular feature correlations were performed to detect possible clustering or epidemiological behaviour. A significance level of 0.05 was used.

III. RESULTS

A. Distrubution of GAS Isolates

The isolates were distributed among the three hospitals in Baghdad City as follows: Medical City Hospital (76 isolates, 43.7%), Yarmouk Teaching Hospital (50 isolates, 28.7%), and Al- Kindi Teaching Hospital (48 isolates, 27.6%). All isolates were confirmed using standard phenotypic and biochemical tests for β-haemolysis, PYR positivity, and bacitracin susceptibility.

B. Distribution of Superantigen and Core Virulence Genes Alongside emm Types

Significant variability was observed in SAg and virulence-associated genes. This variability reflects the different levels of pathogenic potential circulating in paediatric pharyngitis cases. The highest prevalence was observed for the virulence factors scpA (98.3%), sagA (94.3%), slo (97.1%), and nga (95.4%), which were originally part of the PlcR-dependent complete SAg. In contrast, the classical superantigen genes showed the greatest variability, with speA in 26.4% of isolates, speC in 35.6%, and sic in 23.6%. The other genes had intermediate frequencies compared to the three aforementioned genes, with the expressed genes being speB (90.8%), spyCEP (51.1%), cpa/ceuE (87.9%), and fbp54 (75.9%).

A total of 21 distinct emm types were identified during emm gene sequencing. This was with respect to identifiable genetic diversity. The most prevalent of these emm types were emm12 (21.8%), emm22 (17.8%), and emm1 (15.5%). These three types combined accounted for at least 51% of the total isolates. emm63 (6.9%) and emm6 (5.7%) were the least frequent types, while st1815 (1.7%) and stg485 (1.11%) were extremely rare.

TABLE I. SUPERANTIGEN GENE PREVALENCE AND EMM TYPE DISTRIBUTION IN GAS ISOLATES

Category	Gene / emm Type	No. of Isolates	Prevalence (%)
Superantigen / Virulence Genes	emm	174	100.0
	speA	46	26.4
	speB	158	90.8
	speC	62	35.6
	scpA	171	98.3
	sagA	164	94.3
	slo	169	97.1
	spyCEP	89	51.1
	nga	166	95.4
	sic	41	23.6
	cpa/ceuE	153	87.9
	fbp54	132	75.9
emm Types	emm12	38	21.8
	emm22	31	17.8
	emm1	27	15.5
	emm63	12	6.9
	emm6	10	5.7
	st1815	3	1.7
	stg485	2	1.1
	Other 14 emm types	51	29.3

TABLE II. SUPERANTIGEN GENE PREVALENCE AND EMM TYPE DISTRIBUTION IN GAS ISOLATES

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	scpA	171	98.3
	sagA	164	94.3
	slo	169	97.1
	spyCEP	89	51.1
	nga	166	95.4
	sic	41	23.6
	cpa/ceuE	153	87.9
	fbp54	132	75.9
emm Types	emm12	38	21.8
	emm22	31	17.8
	emm1	27	15.5
	emm63	12	6.9
	emm6	10	5.7
	st1815	3	1.7
	stg485	2	1.1
	Other 14 emm types	51	29.3

C. PFGE Analysis and Cluster Distribution

Isolates demonstrated unique patterns of chromosomal SmaI digestion and PFGE fingerprinting. A dendrogram generated using the unweighted pair group method with arithmetic averages (UPGMA) assigned the isolates to 12 PFGE clusters (A-L) at a 0.8 similarity threshold. The cluster distributions were modelled as follows:

TABLE III. DISTRIBUTION OF PFGE CLUSTERS AMONG ISOLATES AND THEIR CORRESPONDING DOMINANT EMM TYPES

PFGE Cluster	No. isolates (%)	Dominant emm Types
Cluster A	29 (16.7%)	emm12, emm1
Cluster B	23 (13.2%)	emm22
Cluster C	18 (10.3%)	emm1
Cluster D	15 (8.6%)	emm6, emm63
Cluster E	14 (8.0%)	emm12
Cluster F	13 (7.5%)	Mixed
Cluster G	12 (6.9%)	emm22
Cluster H	11 (6.3%)	emm63
Cluster I–L	39 (22.4%)	Diverse

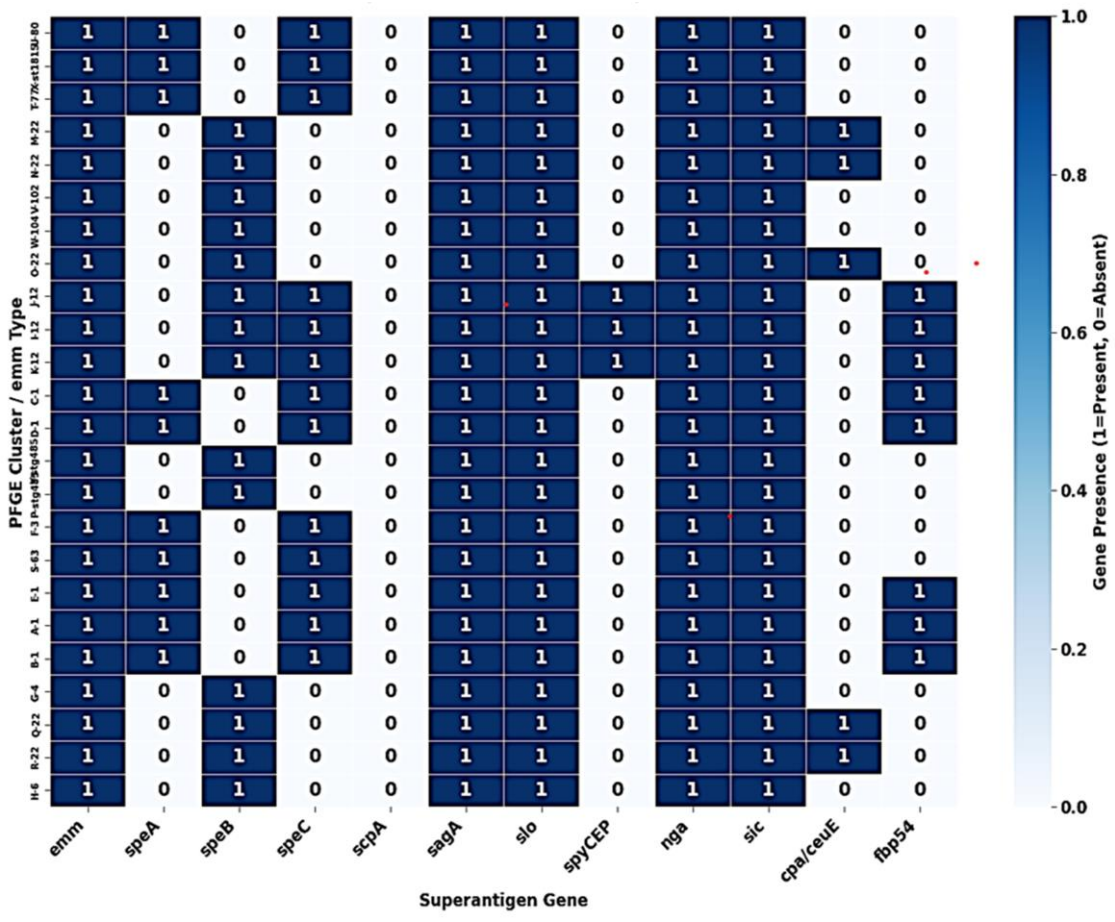

Fig. 1: Superantigen (SAg) gene profile by PFGE cluster and emm type in GAS clinical isolates

D. *Association Between PFGE Clusters, emm Types, and SAg Genes*

PFGE profile, emm type, and superantigen genes resulted in clusters A and B being associated with emm12 and frequently containing speB as well as the genes sagA, slo, nga, and B with predominantly emm22 and frequent speC and spyCEP. Positive strains.

In the majority of emm1 clusters, the C cluster had a markedly high prevalence of speA, reflecting that emm1 clusters with the most speA and high genetic diversity were also those containing speC and sic. Most clusters just had the core virulence genes (scpA, sagA, slo, and nga). It was evident that the distribution was related to the hospitals: Medical City Hospital had the greatest PFGE diversity, Kindi Teaching isolates were mostly in clusters A, B, and D, and Yarmouk Teaching Hospital had a higher number of emm22 and speC-

The dispersal patterns of virulence genes exhibit additional traits linked to differing lineages, as well as a certain degree of likely pathogenicity among circulating strains. For example, a majority (more than 94%) of the core virulence genes, notably scpA, sagA, slo, and nga, were distributed across genetic lineages. Hence, their GAS contributions, GAS fitness, and immune evasion were vital. Meanwhile, the classical superantigens were quite heterogeneous regarding their distribution, specifically with some strains of different emm types; for instance, speA was mostly reported to be present with emm1 strains, while instances of other superantigens, such as speC and sic, were reported to be present at lower frequencies within strains of various emm types.

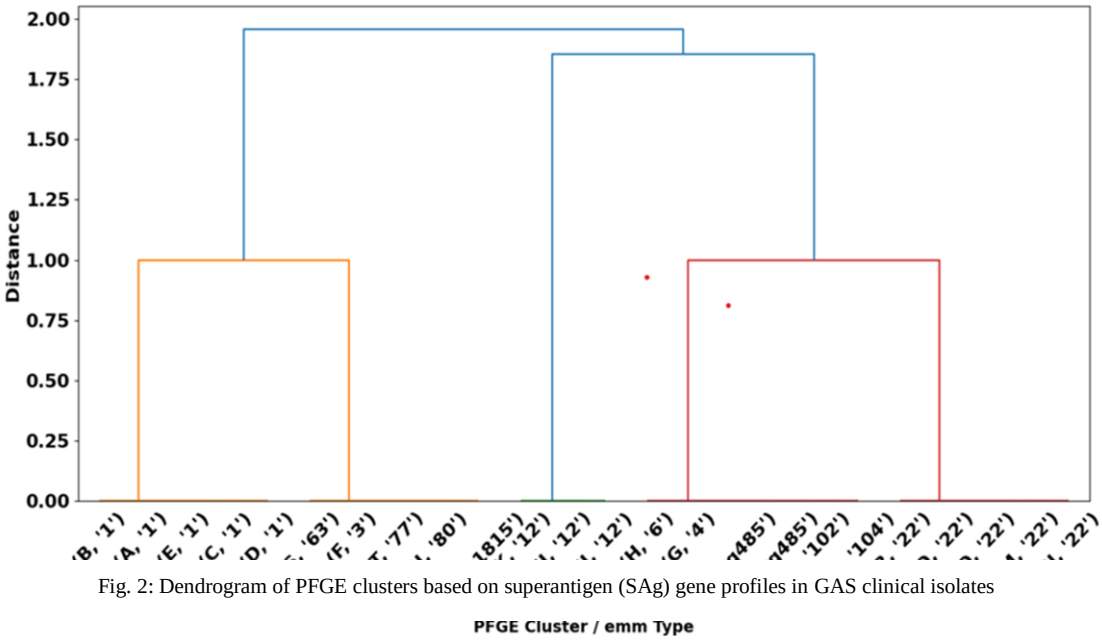

Fig. 2: Dendrogram of PFGE clusters based on superantigen (SAg) gene profiles in GAS clinical isolates

IV. Discussion

This investigation represents the first systematic study of the molecular features of GAS strains causing paediatric pharyngitis among patients in Baghdad City, Iraq. The study reveals a considerable genetic diversity within the population, comprising 174 isolates, among which 21 distinct emm types were detected, with the three main lineages predominating: emm12 (21.8%), emm22 (17.8%), and emm1 (15.5%). These types account for over a third of the population's streptococcal diseases and therefore play an important role in streptococcal disease in the region. The predominance of this triad of emm types reflects the coexistence of universally disseminated epidemic clones and regionally circulating variants, i.e., emm12, emm1, and emm22, suggesting significant circulation and potential local adaptation to host immunity or other conditions in children in Baghdad.

The PFGE (pulsed field gel electrophoresis) data identified 12 primary clonal groups while showing reasonable concordance concerning sub-classifying their emm types and their genomic fingerprints, especially with pity for emm12, emm1 for Cluster A and emm22 for Clusters B and G. The considerable degree of clonality suggests the recent rapid emergence of specific lineages with a fast and efficient spread from one person to another, likely within schools and family groups.

The high prevalence of core virulence genes (scpA, sagA, slo, and nga) in isolates of different emm types is evidence for their fundamental role in the basic pathogenic fitness, as previously reported globally [32- 33].

Certainly, there were striking variations between genotypes: emm1 isolates (15.5%) had the highest rate of speA carriage (81.5% of emm1 isolates carried speA), as might be expected for a superantigen-specific determinant [22]. Conversely, emm22 (17.8%) was strongly associated with

speC (67.7% of all emm22 strains) and spyCEP (58.1%), while association with emm12 (21.8%) was less uniform but was linked to universal core trait genes. These relationships were further supported by PFGE clusters, with cluster A (emm12-dominant) sharing a "stable virulence cassette" of speB+, sagA+, slo+, nga+, and spyCEP+(37), while the uncommon types (e.g., st1815) all had different constellations of other genes.

Our identification of high-level endemicity (21.8% and 15.5%) for emm12 and emm1 is also consistent with the analysis in the Bulgarian population (emm12: 24%, emm1: 18%) [28] as well as the Chinese population (emm12: 19.2%, emm1: 12.5%) [29]. Similarly, our series showed 26.4% of the speA isolates—predominantly within emm1—were comparable to a reported percentage (28%) from Portugal [30] and (25%) from China [31].

Nepal is where [32] reported the second-most-common strain type among school-aged children with pharyngitis. At the same time, [33] reported that the strain type is among the most prevalent among Jordanian isolates. Such findings indicate an emergent clone likely successful in the Eastern Mediterranean Region and adapted to the local host's immunity or conditions. In addition, [34] noted that one of the most prevalent strains associated with invasive infections in North America and Europe likely contributes to the geographical uniqueness of the GAS population in Baghdad, which is, for the most part, characterized by pharyngitis rather than invasive infections.

While disease severity was not systematically recorded, the predominance of types emm1, emm12, and emm22—all previously linked to pharyngitis but also post-streptococcal sequelae such as ARF under endemic conditions [19, 20, 25]—raises public health alarm. Interestingly, in our population, speA, a key superantigen, is frequently carried by emm1 strains. Still, it has to be noted that globally, speA is present in isolates causing invasive disease and ARF [33, 34].

The superantigen gene profile contributes to the virulence traits within specific lineages. The high prevalence of captions (scpA (98.3%), sagA (94.3%), slo (97.1%), and nga (95.4%)) confirms the presence of central virulence factors across multiple emm lineages. The near-total prevalence of these genes has also been reported in India [35] and South Africa [36], suggesting elevated selective pressure for their retention within human-adapted lineages. In contrast to these findings, classical superantigens demonstrated notable diversity; for instance, speA (26.4%) was nearly exclusively found in emm1, in line with the findings from Finland [30] and China [29-31]. Another study found that speA was strongly correlated with the hypervirulent emm1 clonal complex, which is known to cause severe invasive disease.

Every tweaking subsection proportionate statistic gained in proteolysis SpeB = cysteine protease required consort 90.8% S. pyogenes. It is installed implicitly due to the dissemination of SpeB cysteine protease units from the pyogenes strain [37]. Spy CEP fbp54: 51.1% vs. 75.9%; conversely, it might reflect an isolated strain with an embedded proteolytic standstill. Genomic data suggest that GAS community reservoir strains lose esp genes.
In Taiwan, PFGE and emm typing both show strong agreement among the dominant emm strains, such as emm1, emm4, emm6, emm12, and emm22 [38]. The close relationships of emm12 isolates of cluster A may indicate recent clonal expansion, being comparable to the situation in Finland em12, where the breakout caused by this em12 lineage was recorded by [39].

Variability at the hospital level contributes to the overall epidemiological picture. Medical City Hospital has the highest PFGE diversity. This is likely due to Medical City being a tertiary referral hospital that has patients coming from multiple governorates. At the opposite end of the spectrum, Kindi Teaching Hospital and Yarmouk Teaching Hospital had more restrictive strain distributions, with higher proportions of emm22- and speC-positive isolates. This type of inter-institutional diversity has also been observed in multicenter studies from Turkey [40] and Kazakhstan [41], where local antibiotics, infection control practices, and patient demographics determine the circulation of local strains.

The association between PFGE clusters and specific SAg profiles indicates the presence of distinctive, stable virulence gene cassettes in certain clonal lineages. For instance, Cluster A (emm12) always contained in its gene cassettes speB, speC, sagA, slo, spyCEP, nga, sic, and fbp54, which made the combination a stable pathogenic signature. Such stable SAg arrays have also been reported from parts of Japan [42], where some emm types were reported to possess specific gene arrays and to sustain them for decades. By contrast, some rare emm types, for example, st1815 and stg485, have been documented to possess exclusive arrays of their own, which suggests they might be emerging or imported clones.

The variances between Medical City Hospital and the other hospitals in the system have noted the former's greater heterogeneity and diversity of patients and systems, and the wider range of referrals at the tertiary level vs. the uni-level hospitals in the Kindi and Yarmouk hospitals system.
Our isolates were collected from the only 3 largest hospitals in Baghdad, including one national tertiary referral centre, and might not reflect the type and number of healthcare institutions throughout Iraq. As such, these numbers are a snapshot of strains in central Baghdad rather than a national picture at the end of February.

There are, however, some limitations to our study, such as a single-year sampling frame and a complete genomic sequence. Still, it addresses a fundamental question about GAS surveillance in the Middle East. Most reports from Iraq, as discussed above, were limited to phenotypic identification, not to mention molecular details. With the baseline now defined, our study also greatly facilitates surveillance of strain shifts, antimicrobial resistance, vaccine coverage, and, most importantly, GAS within the region.

V. Conclusion

The purpose of this research was to determine the emm types of GAS isolates from children with pharyngitis using SAg gene profiling and PFGE. The most common emm types of S. pyogenes were emm12 (21.8%), emm22 (17.8%), and emm1 (15.5%). Together, they accounted for a little over half of the isolates, in line with the worldwide trend. The analysis also demonstrated a high prevalence of emm22 in this region. No statistically significant differences were observed in the presence of the general virulence genes (scpA, sagA, slo, nga), which were nearly universal (94%-98%), and the classical SAg genes (speA, speC, sic), which were detected at 24%-36%. PFGE demonstrated a very strong correlation with emm types in one of 12 major clonal clusters. For clusters A and G, emm12 and emm1 were the predominant types, while emm22 was the primary type for cluster B, where it was also found that emm1 and speA were strongly associated with one another. More unique hospital trends were observed at the Medical City Hospital, a tertiary referral centre, while localised strain enrichment was demonstrated at Kindi and Yarmouk hospitals. The resulting evidence provides the foundation for surveillance of GAS in Iraq, highlighting regionally significant clones and major globally impactful lineages, and driving the need for vaccine candidates that encompass the emm1, emm12, and emm22 genes.

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Conflict of interest

Authors declare that they have no conflict of interest.

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