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Original article

A post-COVID-19 upsurge in Group A *Streptococcus* in the Czech Republic was driven by *emm*1 and *emm*12 with shared virulence factors

Marek Stefan [a,1], Suhanya Prasad [b,1], Ivana Vitkova [c], Elka Nycova [d], Lenka Ryskova [e], Pavla Kucova [f], Lenka Geigerova [g], Denisa Vesela [h], Magda Balejova [i], Natasa Bartonikova [j], Lenka Havlinova [k], Erika Czyzova [l], Jan Kubele [m], Barbora Dratvova [b], Milena Antuskova [b], Jaroslava Zikova [b], Marie Brajerova [b], Otakar Nyc [b], Milan Trojanek [a], Pavel Drevinek [b], Marcela Krutova [b,*]

[a] Department of Infectious Diseases and Travel Medicine, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic
[b] Department of Medical Microbiology, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic
[c] Department of Clinical Microbiology and Immunology, University Hospital Brno, Brno, Czech Republic
[d] Department of Clinical Microbiology, Bulovka University Hospital, Prague, Czech Republic
[e] Department of Clinical Microbiology, University Hospital Hradec Kralove, Hradec Kralove, Czech Republic
[f] Department of Microbiology, Faculty of Medicine, Palacky University, University Hospital Olomouc, Olomouc, Czech Republic
[g] Department of Microbiology, Faculty of Medicine and University Hospital Plzen, Pilsen, Czech Republic
[h] Department of Medical Microbiology, Jindrichuv Hradec Hospital, Jindrichuv Hradec, Czech Republic
[i] Laboratory of Clinical Microbiology, Hospital Ceske Budejovice, Ceske Budejovice, Czech Republic
[j] Department of Medical Microbiology, Tomas Bata Regional Hospital, Zlin, Czech Republic
[k] Department of Clinical Microbiology and Immunology, Regional Hospital Liberec, Liberec, Czech Republic
[l] Microbiology Laboratory, Sumperk Hospital, Sumperk, Czech Republic
[m] Clinical Microbiology and ATB Centre, Na Homolce Hospital, Prague, Czech Republic

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ABSTRACT

Background: In 2023, a significant increase in Streptococcus pyogenes, Group A Streptococcus (GAS) culture positivity was observed in Czech microbiological laboratories. We conducted a multicentre study to obtain epidemiological data and characterise circulating strains.
Methods: Eleven microbiology departments provided data on single-patient GAS-positive cultures from 2017 to 2023. Additionally, 10 consecutive, single-patient GAS isolates from 12 hospitals were submitted in May 2023 for whole-genome sequencing and antimicrobial susceptibility testing.
Results: In 2023, there was a significant increase in GAS-positive cultures compared to the periods of 2017–2019 ($p = 0.002$), 2020–2021 ($p < 0.00001$), and 2022 ($p = 0.001$), with a disproportionate increase in children. Among 120 isolates, 12 different emm types and 16 multi-locus sequence types (STs) were identified, with emm1 (ST28, 35.0 %) and emm12 (STs 36, 101, 242, 1366, 32.5 %) being the most prevalent. Clonal clustering of emm1 and emm12 isolates across different study sites and geographic regions was demonstrated by whole-genome MLST analysis. When searching for shared virulence genes exclusive to emm1 and emm12 but absent in other emm types, immune evasion and colonisation factors (the streptococcal inhibitor of complement-sic gene in emm1, the distantly related sic-drs gene in emm12, and the sda1 gene in both) were identified.
Conclusions: An upsurge in GAS infections, predominantly caused by emm1 and emm12, was identified in the Czech Republic. The combination of shared virulence factors, altered herd immunity and naïve immunity in children, resulting from contact precautions measures during the COVID-19 pandemic, may have contributed to their increased spread.

* Corresponding author at: Department of Medical Microbiology, Second Faculty of Medicine, Charles University and Motol University Hospital, V Uvalu 84, Prague 5 150 06, Czech Republic.
E-mail address: marcela.krutova@lfmotol.cuni.cz (M. Krutova).
[1] These authors contributed equally.

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Introduction

Streptococcus pyogenes (Group A *Streptococcus*, GAS) is a gram-positive bacterium capable of diverse interactions with the human host, ranging from asymptomatic colonization to a wide spectrum of diseases. This spectrum includes but is not limited to tonsillopharyngitis, scarlet fever, erysipelas, cellulitis, invasive infections, and postinfectious sequelae [1].

Historically, longer-term trends and shorter-term fluctuations in the incidence of GAS infections has been attributed to various factors, including changes in living conditions, improved hygiene, the availability of antibiotics (sulfonamides in the 1930s, followed by penicillins and other antibiotic classes from the 1940s onwards) and the emergence of *S. pyogenes* clones with newly acquired or lost virulence factors. For example, the increase in invasive infections (iGAS) during the 1980s and 1990s was linked to the emergence of a pandemic M1 clone, which had acquired multiple virulence factors through successive horizontal gene transfer events [2,3].

Since the 2010s, cases of scarlet fever have increased, often accompanied by a rise in iGAS infections across multiple countries and continents [1]. This upsurge has been predominantly linked to the *emm*1 strains, particularly a newly identified lineage, $M1_{UK}$ [4], though other *emm* types have also been implicated [5–7]. Following a temporary decline in GAS infections during the COVID-19 pandemic (2020–2021), a sharp resurgence, including iGAS cases, was observed in late 2022 and throughout 2023 [8–15].

In the Czech Republic, scarlet fever, erysipelas and streptococcal sepsis (ICD-10 code A40, which includes sepsis caused by GAS, GBS and other streptococci) are notifiable diseases. Compared to 2019–2022 (including the COVID-19 pandemic of 2020–2021), there has been an upsurge in scarlet fever and streptococcal sepsis in 2023, while the number of erysipelas cases remained stable [16] and $M1_{UK}$ was found to be dominant in invasive strains at the beginning of 2023 [17]; yet comprehensive data on overall GAS infections and circulating *S. pyogenes* strains were lacking. To address this gap, we conducted a multicentric study to obtain epidemiological data and characterise the strains currently circulating in the country.

Materials and methods

S. pyogenes *culture positivity*

Eleven microbiology departments in the Czech Republic provided retrospective anonymised data on the single-patient cultures of *S. pyogenes* from January 1, 2017, to December 31, 2023. In addition, 10 consecutive single-patient *S. pyogenes* isolates from twelve hospitals were sent in May 2023 for further characterisation (Supplementary Table 1). The hospital sites represent 10 of 14 Czech regional areas and include both university and secondary care hospitals. *S. pyogenes* isolates were cultured from both inpatients and outpatients.

The statistical significance between groups was assessed using the chi-square test, Fisher's two-sided exact test, or Student's *t*-test, as appropriate. A test value less than or equal to .05 was considered significant.

Characterisation of 120 consecutive S. pyogenes *isolates*

Antimicrobial susceptibility testing to 16 antibiotics was performed using a disc diffusion method on Mueller-Hinton Agar with Horse Blood (Oxoid, UK) at 37°C with 5 % CO_2 and interpreted according to the EUCAST breakpoints (v14.0), except for gentamicin and chloramphenicol, for which susceptibility categorisation breakpoints are not available. Susceptibility to nitrofurantoin was assessed using the EUCAST breakpoint for Group B *Streptococcus* (v14.0). The double-disc diffusion test was used to test the inducibility of clindamycin in erythromycin-resistant isolates.

DNA extraction was performed after thawing the isolates on blood agar (Oxoid, UK), followed by overnight culture at 37°C with 5 % CO_2. Subculturing was then performed on chocolate blood agar (Oxoid, UK) after a 48-h culture under the same conditions. DNA was purified using the MasterPure™ Complete DNA & RNA Purification Kit (Biosearch Technologies, UK). Genomic DNA was sequenced on the NovaSeq 6000 or NextSeq 2000 using the Nextera XT library preparation kit (Illumina, USA). Raw sequence reads were trimmed to remove adaptors and low-quality bases using fastp v0.24.0 [18] with default parameters. Trimmed reads were then used to perform de novo assembly using Unicycler v0.5.0. Whole genome assembly contigs were annotated using RAST with default settings (https://rast.nmpdr.org/).

The multi-locus sequence type (MLST) and *emm* type were determined using the PubMLST database for *S. pyogenes* [19]. Antimicrobial resistance genes/mutations were detected using ResFinder v4.1 [20], and corresponding genetic mobile genetic elements were predicted using Mobile Element Finder v1.0.3 [21].

Genetic relatedness among isolates was determined using whole genome MLST (wgMLST) with Bionumerics v8.1 (1998 loci, bioMérieux, France).

To investigate the genetic relatedness of Czech *S. pyogenes* isolates within a broader phylogenetic context, we compared them with publicly available genome data for *emm*1 and *emm*12 isolates from 17 countries on four continents (Supplementary Table S2). Phylogenetic trees were constructed using the following approach: first, raw sequence reads were trimmed with fastp v0.24.0, followed by SNP calling, which was performed by mapping the trimmed reads to reference genomes for *emm*1 (MGAS2221, GenBank CP043530) and *emm*12 (MGAS9429, GenBank NC_008021) using Snippy v4.6.0 (https://github.com/tseemann/snippy). The resulting core-genome SNP alignment, with recombinant regions removed using Gubbins v3.3.1 (https://github.com/nickjcroucher/gubbins), was then used to generate maximum likelihood phylogenetic trees. These trees were constructed using the general time-reversible (GTR) model of nucleotide substitution, implemented in RAxML v8.2.12 (https://github.com/stamatak/standard-RAxML) with 100 bootstrap replicates. Additionally, RhierBAPS [22] in R v4.2.3 was used to analyse core-genome SNP alignments and infer population structure through hierarchical Bayesian analysis of population structure (BAPS). Tree visualisation and refinement were performed using iTOL v 6.9.1 [23] and Inkscape v1.3.2.

Virulence-related determinants were predicted using ABRicate v1.0.1 (https://github.com/tseemann/abricate) in conjunction with the Virulence Factor Database (VFDB) [24]. Additionally, we also evaluated the presence of the distantly related *sic* gene (*drs*12.01 allele) because this gene is not included in VFDB. A threshold of 70 % coverage was applied. Allelic variations in the *sic* gene among Czech isolates were analysed using BLASTn against previously published *sic* gene alleles [10]. The prevalence of identified virulence determinants was compared with a global collection of *S. pyogenes* genomes corresponding to *emm*1 (n = 2031) and *emm*12 (n = 1329) from previously published studies (Supplementary Table S2).

Additionally, 27 $M1_{UK}$ lineage-defining single-nucleotide polymorphisms (SNPs) [25] were identified by mapping short reads to the *S. pyogenes* reference genome MGAS5005 (accession number CP000017) [2,3].

Results

Epidemiological trends and shift in GAS culture positivity

A total of 28,977 single-patient *S. pyogenes*-positive cultures were reported from 11 Czech microbiological laboratories between 2017 and 2023 (Fig. 1, Table 1). Of those, 14,313 (49.4 %) were from males and 14,664 (50.6 %) from females. The patient age range was 0–98

Fig. 1. Total GAS culture positivity in 11 Czech hospitals (2017–2023).

years (median = 13, mean = 23.7, interquartile range = 31). Data were available for 28,969 patients, 99.97 %). In 2023, there was a 212 % increase in *S. pyogenes*-positive culture compared to 2022 (p = 0.000158), a 592 % increase compared to the COVID-19 pandemic period of 2020–2021 (p < 0.00001) and a 139 % increase compared to the period preceding the COVID-19 pandemic of 2017–2019 (p = 0.0018) (Table 2).

The increase in *S. pyogenes* culture positivity was not randomly distributed with respect to age. The burden disproportionally occurred in children (0–14 years) when comparing data from 2023 and 2020–2021 (COVID-19 pandemic years), and 2022. A significant increase was also observed in children 5–9 years when comparing data from 2023 and the pre-COVID period, 2017–2019. (Table 3).

S. pyogenes cultures were grouped into the following source categories: blood cultures, ear, eye, throat and nose, lower respiratory tract, skin and soft tissue, purulent specimens (including pus, tissue, and fluid/effusion) and urogenital tract. The source did not differ significantly in 2023 compared to 2017–2019, except for the ear specimens (higher proportion in 2023, p < 0.00001), eye specimens (increase in 2017–2019, p < 0.00001) and throat/nose cultures (increase in 2017–2019, p = 0.0001). Importantly, the proportion of blood cultures, an indicator of invasive and systemic infections, remained relatively stable (1.1 % in 2017–2019 and 1.4 % in 2023, p = 0.1308). Compared to 2023, in 2020–2021, the percentage of ear, throat and nose cultures was significantly reduced, while the proportion of skin and soft tissue and purulent specimen cultures was significantly higher (Table 4).

S. pyogenes *isolates typing and antimicrobial susceptibility testing*

In 120 sequenced GAS isolates prospectively collected from 12 hospitals in 2023, 12 different *emm* types and 16 sequence types (ST)

Table 1
Streptococcus pyogenes culture positivity in 11 Czech hospitals, 2017–2023.

Hospital	2017	2018	2019	2020	2021	2022	2023
A	340	378	309	183	145	315	1051
B	620	376	371	184	126	568	1251
C	224	252	171	112	62	177	549
D	669	599	485	168	68	358	793
E	318	357	311	169	57	275	1138
F	692	754	613	258	177	435	1211
G	373	365	285	172	92	252	874
H	266	285	313	153	92	204	745
I	737	791	737	304	203	492	1712
J	21	116	139	67	39	102	599
K	140	125	124	95	60	60	174
Total	**4 400**	**4 398**	**3 858**	**1 865**	**1 121**	**3 238**	**10 097**

Table 2
Trends in *Streptococcus pyogenes* culture positivity in 11 Czech hospitals, 2017–2023.

Hospital	2023 vs. 2017–2019 Increase by (%)	2023 vs. 2020–2021 Increase by (%)	2023 vs. 2022 Increase by (%)
A	207 %	541 %	234 %
B	175 %	707 %	120 %
C	155 %	531 %	210 %
D	36 %	571 %	122 %
E	246 %	907 %	314 %
F	76 %	456 %	178 %
G	156 %	562 %	247 %
H	159 %	506 %	265 %
I	127 %	574 %	248 %
J	551 %	1030 %	487 %
K	34 %	123 %	190 %
Mean	**139 %**	**592 %**	**212 %**
P-value	**0.0018**	**0.00001**	**0.000158**

were identified, with *emm*1 (n = 42, 35.0 %) and *emm*12 (n = 39, 32.5 %) being the most prevalent. All *emm*1 isolates belonged to the same ST (ST28), whereas *emm*12 isolates belonged to three different STs (ST36 n = 32, ST242 n = 4, ST1366 n = 3). With the exception of one hospital, both *emm* types were detected across study sites, although in varying proportions (Fig. 2a). Among these, 10 were cultured from blood with *emm*1 (n = 6) and *emm*12 (n = 3) as the dominant ones. Other *emm* types represented by more than five isolates included *emm*4 (ST39, n = 10, 8.3 %), *emm*28 (STs 52 n = 4 and 458 n = 3, 5.8 %) and *emm*49.8 (ST190, n = 6, 5 %). A summary of *emm* types is provided in Fig. 2b and the Supplementary Table S1.

Based on the presence of 27 $M1_{UK}$ lineage-defining SNPs, the *emm*1 isolates predominantly belonged to the $M1_{UK}$ lineage (28/42 isolates, 66.7 %), while the remaining 14 isolates (33.3 %) belonged to the $M1_{Global}$ lineage.

All 120 isolates were sensitive to penicillin, ampicillin, cefalotin, cefotaxime, cotrimoxazole, rifampicin, vancomycin, linezolid, tigecycline, nitrofurantoin and gentamicin. Antimicrobial resistance was detected to erythromycin (14.2 %), clindamycin (5.0 %), inducible clindamycin (3.3 %), norfloxacin (2.5 %), chloramphenicol (5.0 %) and tetracycline (15.8 %). Antimicrobial resistance genes found were: *erm*B (n = 6, macrolides, lincosamides), *erm*A (n = 4, macrolides and inducible lincosamides), *msr*D and *mef*A (n = 6, macrolides), *cat*Q (n = 6, chloramphenicol) and *tet* (*tet*M n = 17, *tet*O n = 2, *tet*L n = 1, tetracyclines). The *par*C p.S79F associated with resistance to fluoroquinolones was found in one isolate. The corresponding mechanism of resistance was not identified in GAS304 (erythromycin-resistant, clindamycin-susceptible, non-inducible).

In eleven isolates, the antimicrobial resistance determinants were associated with the Tn*6009* transposon, and in three isolates with the ISSpy1 insertion element. In five isolates, the corresponding mobile element was not identified (Supplementary Table S1).

Dominance of emm*1 and* emm*12 in the Czech Republic and Europe*

The wgMLST analysis revealed clustering based on *emm* type, with *emm*1 isolates displaying greater clonal relatedness (0–2 allele differences) compared to *emm*12 isolates (Fig. 3a). The clonal clustering of isolates with the same *emm* type was also observed across different study sites and geographic regions (Fig. 3b).

Maximum likelihood phylogeny of *emm*1 and *emm*12 isolates showed that Czech *S. pyogenes* isolates did not form a single monophyletic cluster, indicating they are not derived from one locally expanding lineage. Instead, Czech isolates were interspersed among strains from various other countries, suggesting multiple independent introductions.

$M1_{UK}$ Czech isolates clustered with isolates from Belgium, Portugal, Spain and the UK. Similarly, $M1_{Global}$ isolates were closely related to those from Belgium, Spain, Denmark, and Australia (Fig. 4).

Table 3
Culture positivity rate in different age groups. *In 2022, gender data was not available for 1 individual, and in 2023 for 6 individuals. **Red: 2023 significantly higher than years compared, green: 2023 significantly lower than years compared, black: not significant.

Age (years)	2017-2019 (mean No. of cultures/year)	2020-2021 (mean No. of cultures/year)	2022 (No. of cultures/year)	2023 (No. of cultures/year)	2023 versus 2017-2019 (p values) **	2023 versus 2020-2021 (p values) **	2023 versus 2022 (p values) **
0-4	456	100	303	1180	0.1341	< 0.00001	0.0002
5-9	1144	235	938	3541	< 0.00001	< 0.00001	< 0.00001
10-14	541	131	324	1276	0.7621	< 0.00001	0.0001
15-19	207	104	194	409	0.0239	< 0.00001	< 0.00001
20-24	147	77	119	233	0.0001	< 0.00001	0.0001
25-29	184	91	140	286	< 0.00001	< 0.00001	0.0001
30-34	239	122	216	542	0.4927	0	0.006
35-39	342	132	225	693	0.0098	0.0082	0.8732
40-44	272	121	180	559	0.0377	0.0015	0.9648
45-49	143	72	143	348	0.88	0.0115	0.0135
50-54	112	69	81	206	0.0251	< 0.00001	0.1256
55-59	90	55	87	172	0.0872	< 0.00001	0.0007
60-64	104	47	67	186	0.019	0.0015	0.4157
65-69	73	43	68	162	0.6136	0.0011	0.0628
70-74	64	36	66	130	0.3026	0.0015	0.0032
75-79	48	28	39	90	0.1887	0.0013	0.1216
80-84	22	16	20	42	0.4102	0.0025	0.1402
85- 89	17	10	14	24	0.1208	0.0088	0.0865
90-94	10	6	10	11	0.0908	0.0163	0.0197
95-99	3	2	3	1	0.0798	0.0457	0.0468
Total	4218	1497	3237*	10091*	Fisher exact test		

Table 4
GAS culture positivity at different culture sites, 2017–2019 = " pre-SARS-CoV-2 years", 2020–2021 = " SARS-CoV-2 years", statistical difference between 2017 and 2019 and 2023 is shown. *Culture site location not available for 1 individual. **Red: 2023 significantly higher than years compared, green: 2023 significantly lower than years compared, black: not significant.

Culture sites	2017-2019 (mean No. of cultures/year)	2020-2021 (mean No. of cultures/year)	2022 (No. of cultures/year)	2023 (No. of cultures/year)	2023 versus 2017-2019 (p value) **	2023 versus 2020-2021 (p value) **	2023 versus 2022 (p value) **
Blood culture	47	35	61	146	p=0.1308	p=0.1114	p=0.0861
Ear	176	39	196	844	p<0.00001	p<0.00001	p=0
Eye	12	5	6	25	p<0.00001	p=0.581	p=0.6758
Lower respiratory tract	51	23	32	114	p=0.6686	p=0.198	p=0.4998
Skin and soft tissue	693	512	873	1499	p=0.2371	p<0.00001	p<0.00001
Purulent samples	142	93	178	380	p=0.2608	p=0	p=0
Throat/nose	2949	740	1790	6705	p=0.0001	p<0.00001	< 0.00001
Urogenital tract	150	48	102	383	p=0.5291	p=0.3049	p=0.0944
Total	4220	1495	3238	10096*	Fisher exact test		

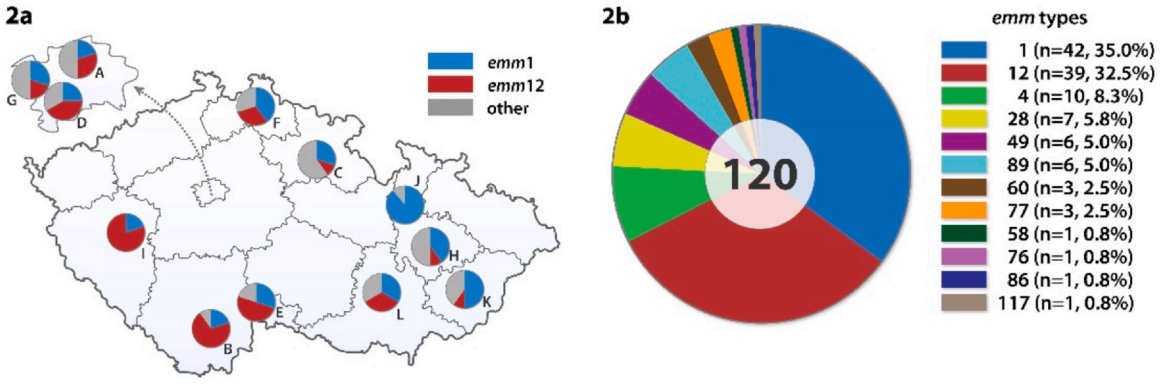

Fig. 2. a: The distribution of *emm*1 and *emm*12 in twelve hospitals (A-L) in the Czech Republic. b: *S. pyogenes emm* types identified in consecutive isolates from twelve hospitals in the Czech Republic.

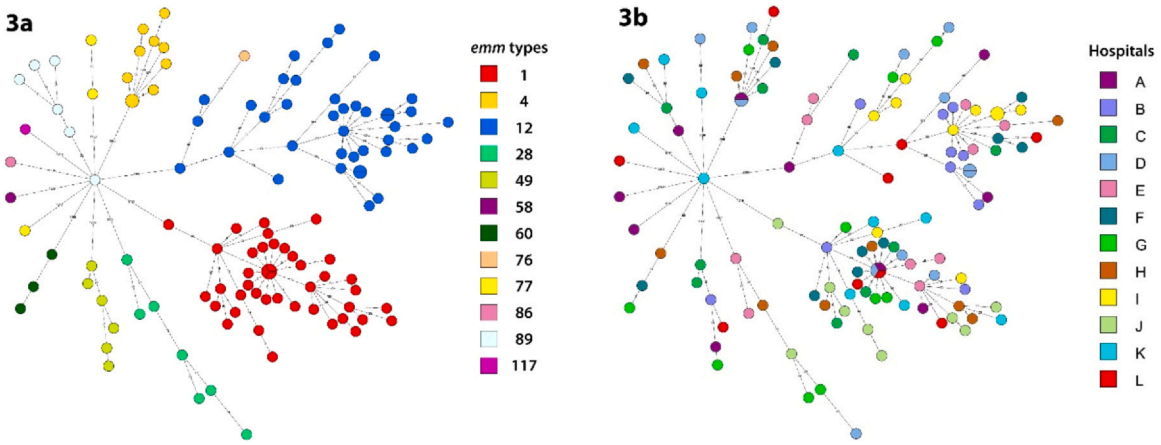

Fig. 3. a: Minimum spanning tree from wgMLST analysis of *Streptococcus pyogenes* isolates (n = 120) according to *emm* type and 3b) Minimum spanning tree from wgMLST analysis of *Streptococcus pyogenes* isolates (n = 120), according to hospital (A-L).

For *emm*12 isolates, hierBAPS population-level clustering identified five groups, and the majority of Czech isolates belonged to cluster 2 (n = 24) and cluster 3 (n = 12), clustering alongside isolates from the UK, Spain, Denmark, and the USA. Only two Czech isolates from throat samples were found separately in cluster 1, closely related to isolates from Spain and the UK, while a single isolate was identified in cluster 4 (Fig. 5).

Virulence factors shared by emm*1 and* emm*12*

The distribution of virulence factors was largely *emm*-type specific (Supplementary Table S3). Overall, their frequencies were comparable to those observed in the global genome dataset, with a few exceptions. In *emm*1 isolates, the *spe*C, *hyl*P, and *mf*2/*spd*2 genes were not consistently present in all Czech or global strains. Moreover, in *emm*12 isolates, *spe*C and *mf*2/*spd*2 were detected at a lower frequency in Czech strains compared to the global dataset (Supplementary Tables S3 and S4).

Unique virulence determinants to *emm*1 were the *fct*A and *fct*B genes, which encode GAS pilin proteins and co-exist with the *lep*A gene, functioning as a signal peptidase I, in the fibronectin-collagen T-antigen (FCT) region. These genes are involved in host adherence and the formation of biofilms. In addition, the *srtc*1 gene, located in the same region, was also found in *emm*1 isolates, although at a low frequency (2.56 %) in other *emm* types. In contrast, the *spe*H and *spe*I exotoxin genes were detected exclusively in *emm*12 isolates (Supplementary Table S3).

A search for virulence factors shared by *emm*1 and *emm*12 isolates identified the streptodornase gene (*sda*1), along with two related genes: the *sic* gene, present in *emm*1, and the distantly related *sic* gene (*drs*12.01 allele), present in *emm*12 isolates.

Comparison with *sic* allele sequences published by Beres *et al.* identified seven *sic* alleles with 100 % similarity and coverage in 34 out of 42 *emm*1 isolates. The majority of $M1_{UK}$ isolates (n = 18/28) carried the *sic*-0001 allele (933 bp), while $M1_{Global}$ isolates (5/14) carried the *sic*-0012 allele. Eight isolates showed less than 100 % similarity with published allele sequences [10]. The distribution of individual *sic* alleles is presented in Supplementary Table S5.

In Czech *emm*12 isolates, all *drs* gene sequences showed 100 % similarity to the *drs*12.01 allele [26].

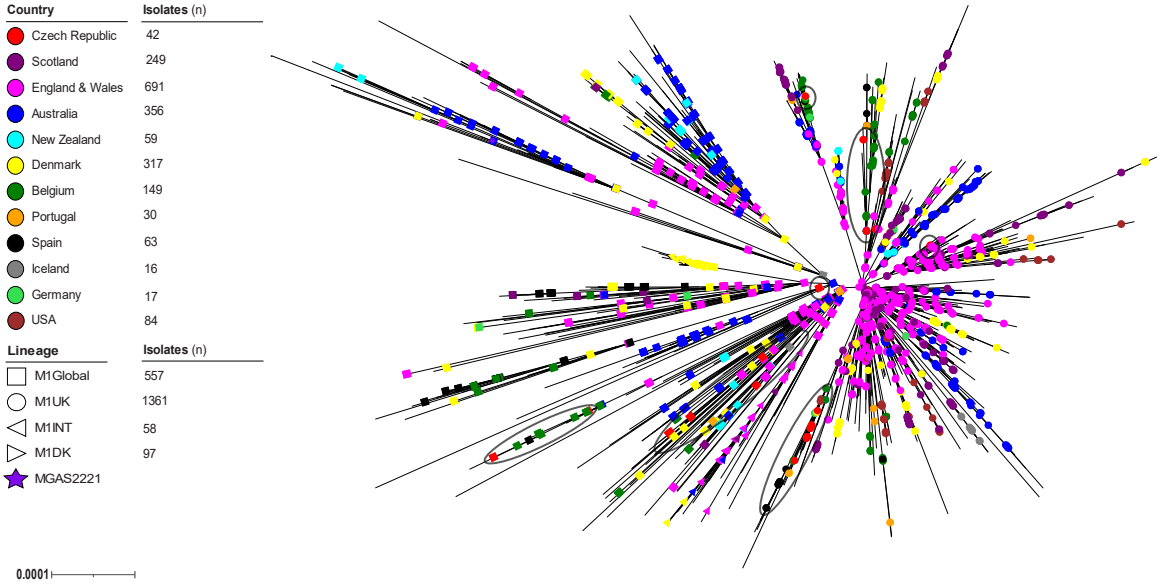

Fig. 4. An unrooted maximum-likelihood phylogenetic tree for 2073 *emm*1 isolates, including 42 *emm*1 strains from the Czech Republic collected in 2023, was inferred based on 8953 core chromosomal SNPs identified by mapping Illumina short reads to the reference emm1 strain MGAS2221 (GenBank CP043530). The scale bar represents the number of nucleotide substitutions per site. The clusters of Czech and European isolates are highlighted in the elliptical shapes.

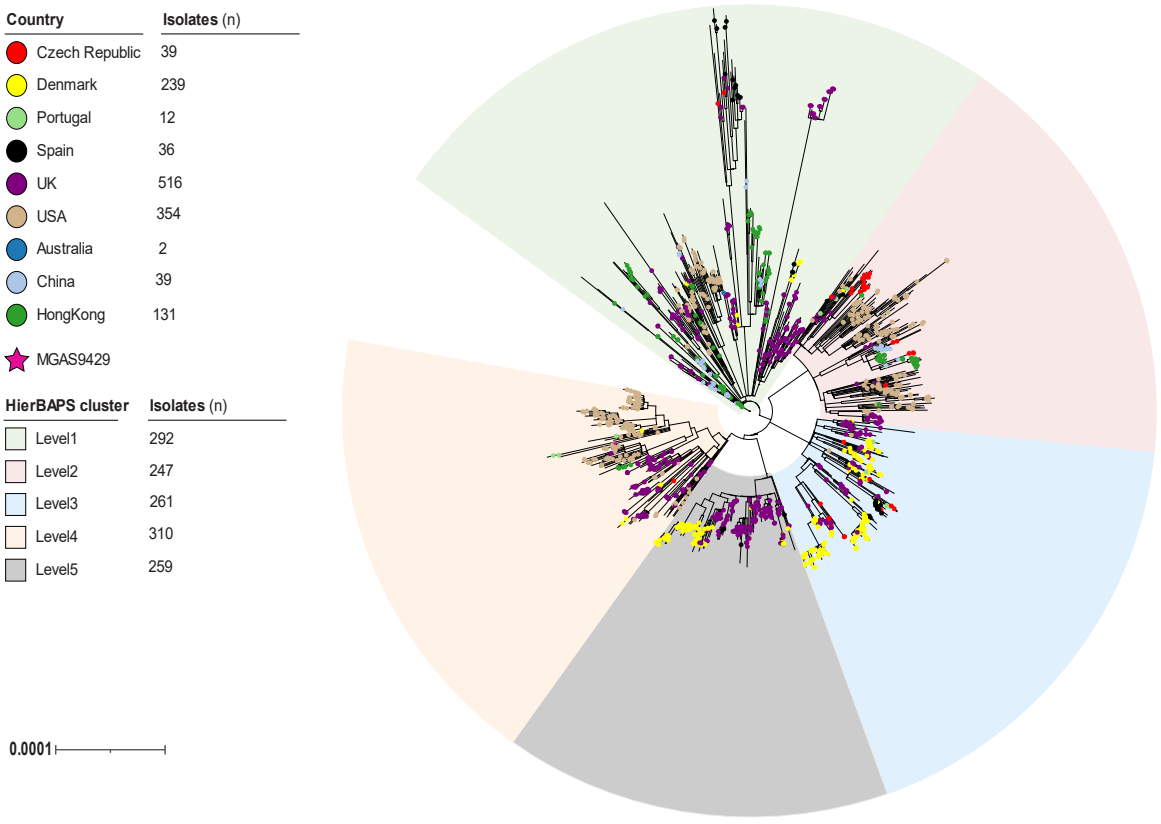

Fig. 5. A maximum-likelihood phylogenetic tree for 1368 *emm*12 isolates, including 39 *emm*12 isolates from the Czech Republic collected in 2023, was inferred based on 6442 core chromosomal SNPs by mapping Illumina short-reads to the reference *emm*12 strain MGAS9429 (GenBank NC_008021). The hierBAPS top-level cluster assignments are denoted in different colours. The scale bar represents the number of nucleotide substitutions per site.

Discussion

A global increase in GAS infections, including invasive cases, was observed in late 2022 and throughout 2023, with the $M1_{UK}$ subclone being numerically predominant in multiple countries, especially in Europe [8–15,27]. The exact reason behind this increase remains unclear. One possible explanation is reduced exposure to respiratory pathogens during the COVID-19 pandemic, which may have led to increased susceptibility to respiratory infections. Additionally, a shift in the epidemiology of circulating strains may have also contributed to this change. In the Czech Republic, comprehensive data on overall GAS infections and circulating *S. pyogenes* strains have been lacking.

To address this gap, we initiated a multicentre study in May 2023. Data from 11 hospitals showed a significant increase in GAS culture positivity in the Czech Republic from 2017 to 2023, with paediatric age groups disproportionately affected. The rise in GAS infections following the COVID-19 pandemic can largely be attributed to changes in herd immunity, resulting from non-pharmacological interventions, such as mask mandates, school and preschool closures, and reduced interpersonal contact, which suppressed the transmission of respiratory pathogens [28,29]. Notably, a shortage of β-lactam antibiotics, particularly paediatric formulations, across the EU, including the Czech Republic, in 2023, may have further influenced transmission dynamics [30,31].

Culture positivity across sample locations remained largely stable over the seven-year period, except for an increase in upper respiratory cultures in 2023, compared to 2020 – 2022. During the pandemic, non-respiratory samples (e.g., purulent specimens and samples from skin and soft tissue infections) were more frequently cultured. Together, these findings may reflect reduced respiratory droplet transmission and ongoing contact transmission of GAS during the pandemic. Importantly, the proportion of positive blood cultures remained stable, indicating that iGAS infections (primarily detected through blood cultures) did not rise disproportionately compared to non-invasive infections.

Considering the *emm* type distribution, our study identified *emm*1 (35 %) and *emm*12 (32.5 %) as the dominant *emm* types in 2023, which is consistent with trends reported in Denmark [12], Portugal [14], Spain [15] and Germany [32]. Phylogenetic clustering of Czech isolates with strains from other European countries suggests multiple independent introductions of these lineages into the Czech Republic.

In our study, the majority of isolates were non-invasive; however, 10 were cultured from blood and thus classified as invasive. No differences in virulence factors were found between invasive and non-invasive isolates of the same *emm* type. When searching for shared virulence patterns exclusive to *emm*1 and *emm*12 but absent in other *emm* types, an immune evasion factor was identified in both *emm*1 and *emm*12 isolates, encoded by two distinct genes (the *sic* gene in *emm*1 and the *drs* gene in *emm*12). Additionally, both *emm* types harboured the *sda*1, streptodornase gene.

The *sic* gene encodes a streptococcal inhibitor of complement, a protein that binds to and inactivates components of the innate immune response, including the C5b67 complex, lysozyme, α- and β-defensins, secretory leukocyte protease inhibitor, LL-37, and histones, while also promoting bacterial adherence to epithelial cells [33–35]. Notably, distinct *sic* gene variants were identified among *emm*1 isolates, supporting previous findings that epidemic waves of M1 strains consist of multiple subclones, each carrying different *sic* variants undergoing rapid selection [36,37].

The *drs* (distantly related to SIC) gene encodes a protein that binds to C6 and C7 complement proteins and inhibits the antimicrobial activity of LL-37 [38,39]. Both SIC and DRS suppress other antimicrobial peptides (AMPs), further enhancing bacterial

colonisation [40,41]. This inhibitory function is believed to provide a significant survival advantage to *emm* types carrying these genes [42]. Unlike the hypervariable *sic* gene, all the *drs* gene alleles in our study were identical.

Streptodornase (*sda*1) is a DNase that degrades neutrophil extracellular traps (NETs), which are essential for bacterial clearance by neutrophils [43]. The ability to produce streptodornase may provide a selective advantage by immune evasion, bacterial fitness, and virulence [44,45].

The virulence factors mentioned above are well-established features of M1 and/or M12 strains. Their presence likely contributes to the high prevalence of these types in GAS infections overall. Combined with reduced herd immunity, this may help explain their predominance during the post-COVID-19 surge.

Conclusion

An upsurge in GAS infections, driven by the predominance of *emm*1 and *emm*12, was identified in the Czech Republic in 2023. The findings align with trends observed across Europe, with phylogenetic analysis confirming a close genetic relationship between Czech and European isolates. The presence of shared virulence factors highlights their potential role in the persistence and pathogenicity of these strains. Additionally, non-trained immune response in children due to contact precautions measures during the COVID-19 pandemic may have contributed to their increased spread. Continued genomic surveillance is important for tracking the evolution of *S. pyogenes* and its impact on public health.

Ethics

The project was approved by the local ethics committee at the University Hospital Motol, Prague, Czech Republic (Reference No. EK-578/23).

The authors' contribution

MS, MK – data analysis, study design, co-drafting of the first version of the manuscript and editing its subsequent versions, SP – data analysis, study design, co-drafting of the first version of the manuscript and editing its subsequent versions, IV, EN, LR, PC, LG, DV, MBa, NB, LH, EC, JK – collection of strains, editing the subsequent versions of the manuscript, BD, MA, JZ, MB – strain culture, antimicrobial susceptibility testing, DNA extraction, library preparation, bioinformatic analysis, editing the subsequent versions of the manuscript, ON, MT – microbiological data analysis, editing the subsequent versions of the manuscript, PD – study design, editing subsequent versions of the manuscript, funding acquisition.

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Data availability

Raw sequence data generated in this study are deposited in the Sequence Read Archive under BioProject: PRJNA1255298.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jiph.2025.102886.

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