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Original Article

Distribution of *emm* genotypes in group A streptococcus isolates of Korean children from 2012 to 2019

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Abstract *Objectives:* Changes in the epidemiology of group A streptococcus (GAS) infection is related to *emm* genotype. We studied the distribution of *emm* genotypes and their antibiotic susceptibility among Korean children.
Methods: Isolates from children with GAS infection between 2012 and 2019 were collected. *emm* typing and cluster analysis was performed according to the Centers for Disease Control *emm* cluster classification. Antimicrobial susceptibility was tested using the E-test and resistance genes were analyzed for macrolide resistant phenotypes.
Results: Among 169 GAS isolates, 115 were from children with scarlet fever. Among invasive isolates, *emm*1 (6/22, 27.3%), *emm*12 (4/22, 18.2%), and *emm*4 (4/22, 18.2%) were most common. In scarlet fever, although *emm*4 (38/115, 33.0%) was the most prevalent throughout the study period, *emm*4 was replaced by *emm*3 (28/90, 31.1%) during an outbreak in 2017—2018. Among all isolates, only 2 (1.2%) exhibited erythromycin resistance and harbored both *ermA* and *ermB* genes.
Conclusions: In this analysis of GAS isolated from Korean children, *emm*1 was the most prevalent in invasive infection. In scarlet fever, *emm*4 was prevalent throughout the study period,

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with an increase in *emm*3 during 2017—2018. GAS isolates during 2012—2019 demonstrated low erythromycin resistance.

Introduction

Group A streptococcus (GAS) infection is one of the most common bacterial diseases in children. It has a wide range of clinical presentations from mild diseases such as pharyngitis and impetigo to severe complications including rheumatic fever and post-streptococcal glomerulonephritis (PSGN).[1,2] Invasive GAS diseases, including streptococcal toxic shock syndrome (STSS) and necrotizing fasciitis, exhibit a significant clinical burden in children and could be life-threatening.[3]

Streptococcal M protein, a surface protein of GAS, is a major virulence factor[4—6] allowing adherence to host tissue and promoting bacterial colonization.[5,6] While the C-terminal region of the M protein is conserved in various strains, the N-terminal region is hypervariable, which contributes to the diversity of the GAS serotypes.[6] The M protein is encoded by the *emm* gene, and the prevalence of GAS *emm* genotypes differs geographically and over periods of time.[2] Currently, >200 *emm* genes have been identified, and 48 *emm*-clusters are used to classify these functionally.[7,8] Changes in *emm* genotype are related to the changes in the epidemiology of GAS infection.

In this study, we analyzed the distribution of *emm* types and gene clusters based on the clinical manifestations in Korean children diagnosed with GAS infections from 2012 to 2019. In addition, we tested the antibiotic susceptibility to investigate and identify antibiotic resistance genes prevalent in the study population.

Methods

Study design

GAS isolates from Korean children ≤18 years of age, who presented to 7 different hospitals between 2012 and 2019 were collected: Seoul National University Children's Hospital, Seoul National University Bundang Hospital, Chungnam National University Hospital, Gachon University Gil Medical Center, Samsung Changwon Hospital, Daejeon St. Mary's Hospital, and Pusan National University Yangsan Hospital. Medical records were reviewed; demographic information of patients including age, sex, clinical diagnoses, and underlying diseases was collected. The clinical diagnoses included pharyngitis, scarlet fever, otitis, noninvasive skin and soft tissue infection (SSTI), and invasive diseases. Invasive diseases were defined as cases in which GAS was isolated from sterile body fluids, and those with STSS or with necrotizing fasciitis. The study was carried out in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of the Seoul National University Bundang Hospital (B-2102-664-302). Written informed consent was waived in this study.

GAS identification

GAS isolates were identified using the following methods; β-hemolysis in 5% sheep blood agar, bacitracin susceptibility test, and latex fixation test.[9] The test for bacitracin susceptibility was performed using a disk with a concentration of 10 U; the diameter of the inhibition zone was measured. Latex fixation test, also known as latex agglutination assay, was performed to differentiate among group A, B, C, and G streptococci.

Antibiotic resistance and related genes

We tested the GAS isolates for antibiotic susceptibility for penicillin, erythromycin, and clindamycin. The minimal inhibitory concentration (MIC) was measured, and the cut-off point was determined according to the 2019 Clinical and Laboratory Standards Institute (CLSI) guidelines. GAS isolates with macrolide resistance were classified as $cMLS_B$ (constitutive macrolide, lincosamide, streptogramin B: both resistant to erythromycin and clindamycin), $iMLS_B$ (inducible), and M (resistant to erythromycin only) phenotypes.[10]

GAS isolates resistant to erythromycin were further investigated for the presence of *erm*A, *erm*B, and *mef*A genes using polymerase chain reaction (PCR), as described previously.[11—16]

emm typing

DNA was extracted from GAS isolates and amplified using PCR. The reaction mix consisted of 2 μl 10X buffer, 1.6 μl $MgCl_2$, 1.6 μl dNTP, 0.15 μl Taq polymerase, 0.2 μl primer-S, 0.2 μl primer-AS, 3 μl DNA, and 11.25 μl dh20. The reaction conditions were as follows: 96 °C (1 min), 25 cycles at 96 °C (10 s), 55 °C (5 s), 60 °C (4 min), and 4 °C (holding). The sequence of primers 1 and 2 are ATTCGCTTAGAAAATTAAAAACAGG and GCAAGTTCTTCAGCTTGTTT, respectively. The sequence analysis software, Sequencher® was used to compare the *emm* gene sequences among the isolates. Analysis and classification of *emm* genotypes and clusters was performed with reference to the *emm* cluster database of the Centers for Disease Control and Prevention (CDC, https://www2.cdc.gov/vaccines/biotech/strepblast.asp).

Estimates of vaccine coverage

We compared the *emm* types sequenced from the isolates with that included in the proposed 30-valent GAS vaccine. The vaccine was to include *emm*1, *emm*2, *emm*3.1, *emm*4, *emm*5.14, *emm*6.4, *emm*11, *emm*12, *emm*14.3, *emm*18, *emm*19, *emm*22, *emm*24, *emm*28, *emm*29.2, *emm*44, *emm*49, *emm*58, *emm*73, *emm*75, *emm*77, *emm*78, *emm*81, *emm*82, *emm*83.1, *emm*87, *emm*89, *emm*92, *emm*114, and *emm*118 type gene sequences.[17]

Statistical analysis

The chi-squared test and Fisher's exact test were used to analyze categorical variables. The IBM SPSS® software, version 26 was used for data analysis. A p-value of <0.05 was considered to denote statistical significance.

Results

Demographic features and clinical presentations

A total of 169 GAS isolates were included in this study. The median age of patients was 6 ± 6.6 years (range, 0–16 years). The age group with the highest prevalence of GAS infection was 4–6 years accounting for 53.8% (n = 91). Comparing the age groups according to the diagnosis, the proportion of patients <2 years of age was higher among those diagnosed with invasive disease (27.2%) compared to that among those with non-invasive (1.3%) GAS infection ($p < 0.001$) (Fig. 1).

The patients were categorized into 2 groups, invasive and non-invasive, based on the clinical presentation and were further subcategorized into 12 different diseases (Table 1). Among the patients with invasive infections (n = 22), invasive SSTI (31.8%, n = 7) was the most prevalent clinical manifestation followed by bacteremia (27.2%, n = 6) and bone and joint infection (13.6%, n = 3). Among the patients with non-invasive infections (n = 147), scarlet

Table 1 Characteristics of children diagnosed with Group A Streptococcal infection.

Characteristics	N (N = 169)	Percentage
Age (y) (Mean ± SD)	6 ± 3.32	
Sex (male)	99	58.6
Underlying disease		
Endocrine disease[a]	1	0.6
CNS disease[b]	2	1.2
Respiratory disease[c]	1	0.6
Site of isolation		
Blood	11	6.5
Throat	133	78.7
Otorrhea	5	3
Skin and soft tissue[d]	16	9.5
Bone and joint fluid	3	1.8
BAL fluid	1	0.6
Clinical presentation		
Non-invasive	147	87
Pharyngitis	13	7.7
Scarlet fever	115	68
Ear infection	5	3
SSTI	14	8.3
Invasive	22	13
Bacteremia	6	3.6
STSS	1	0.6
Deep neck infection	1	0.6
Bone and joint infection	3	1.8
Lung infection	2	1.2
CNS infection	1	0.6
Infective endocarditis	1	0.6
Invasive SSTI	7	4.1

[a] Congenital adrenal hyperplasia.
[b] Encephalitis, CSF rhinorrhea.
[c] Pierre Robin Syndrome and tracheomalacia.
[d] Skin and pus swab, pus aspiration; SD standard deviation, CNS central nervous system, BAL bronchoalveolar lavage, SSTI skin and soft tissue infection, STSS streptococcal toxic shock syndrome.

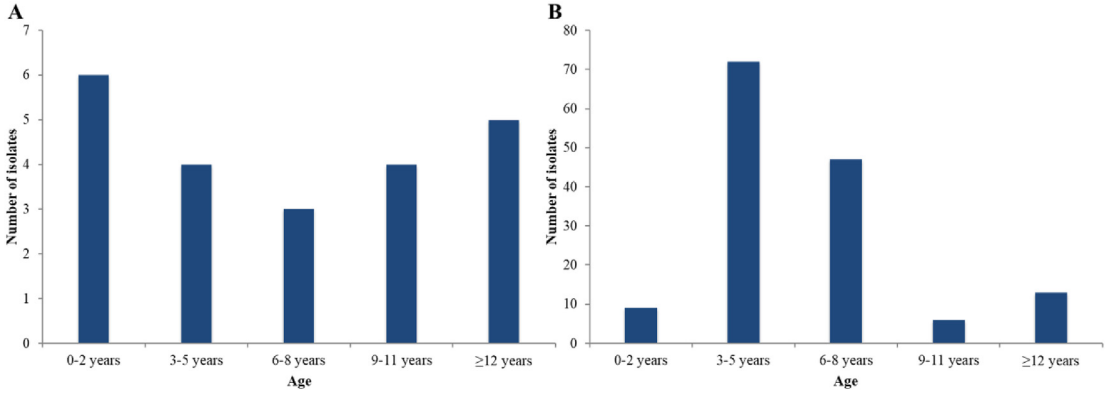

Figure 1. Distribution of isolates according to patient age in invasive and non-invasive infections. (A) Invasive infections, (B) non-invasive infections.

fever was the most common disease (78.2%, n = 115) followed by non-invasive SSTI (9.5%, n = 14), pharyngitis (8.8%, n = 13), and ear infection (3.4%, n = 5). In particular, scarlet fever accounted for 68% (n = 115) of all GAS isolates.

M protein genotypes (*emm* types)

Among all isolates, we identified 10 different *emm* types. Among these, *emm*4 (30.2%), *emm*3 (18.9%), *emm*1 (12.4%), and *emm*12 (10.1%) were the most prevalent genotypes. When applying functional classification of *emm* genotypes, all isolates were classified into 8 *emm* clusters among which E1 (30.2%), A-C5 (18.9%), E4 (15%), A-C3 (12.4%), and A-C4 (10.1%) were common (Table 2).

Among the invasive infections, *emm*1 (6 cases, 27.3%) was most prevalent, followed by *emm*12 (4 cases, 18.2%), *emm*4 (4 cases, 18.2%), *emm*6 (2 cases, 9.1%), and *emm*28 (2 cases, 9.1%) (Fig. 2).

Among the non-invasive infections, *emm*4 (32.0%), *emm*3 (21.1%), *emm*1 (10.2%), and *emm*12 (8.8%) were most common and the distribution differed between diseases.

Among the non-invasive SSTI, *emm*4 (35.7%) and *emm*12 (21.4%) were the most prevalent genotypes. The distribution in non-invasive SSTI differed significantly from that in invasive SSTI ($p < 0.001$), in which *emm*6 (28.6%) and *emm*28 (28.6%) were commonly found.

Among 115 patients with scarlet fever, ninety cases (90/115, 78.2%) were collected during an outbreak in 2017–2018. During the total study period, *emm*4 was the most common genotype (33%, n = 38), followed by *emm*3 (25.2%, n = 29) in scarlet fever. However, when comparing the distribution in 2012–2016 and 2017–2018, *emm*4 decreased from 58.3% to 25.6% in all cases. In 2017–2018, *emm*3 was the most common type at 31.1% followed by *emm*4 at 25.6% (Fig. 3). Genotypes *emm*1 (4 cases, 30.8%), *emm*4 (3 cases, 23.1%), *emm*12 (2 cases, 15.4%), and *emm*28 (2 cases, 15.4%) were commonly identified from children with pharyngitis.

To evaluate the possible coverage of vaccine for the various *emm* types, we compared *emm* types identified from all 169 isolates to those proposed to be included in the 30-valent GAS vaccine. We found that all *emm* types detected during the study period were covered by the 30-valent vaccine.

Table 2 *emm* cluster in isolates from children with Group A Streptococcus infections in Korea from 2012 to 2019.

emm cluster	*emm* type	N (%)
A-C3	*emm* 1	21 (12.4)
A-C4	*emm* 12	17 (10.1)
A-C5	*emm* 3	32 (18.9)
M6	*emm* 6	11 (6.5)
E1	*emm* 4	51 (30.2)
E3	*emm* 87	1 (0.6)
E4	*emm* 28, *emm* 89	27[a] (16)
E6	*emm* 11, *emm* 75	9[b] (5.3)

[a] *emm* 28 (n = 14), *emm* 89 (n = 13).
[b] *emm* 11 (n = 1), *emm* 75 (n = 8).

Antibiotic resistance

All GAS isolates were sensitive to penicillin. In total, 2/169 (1.2%) isolates demonstrated macrolide resistance. The MIC of erythromycin and clindamycin determined in both resistant samples were 256 μg/mL, respectively. Patients infected by macrolide resistant strains were diagnosed with pharyngitis or scarlet fever. M protein genotypes of these strains were *emm*28 and *emm*12, classified as *emm* cluster E4 and A-C4, respectively. Both macrolide resistant isolates expressed the $cMLS_B$ phenotype and demonstrated resistance to both erythromycin and clindamycin. Further, both isolates harbored *erm*A and *erm*B genes.

Discussion

In this study, we investigated the distribution of *emm* type genes and antimicrobial susceptibility in GAS strains isolated from Korean children between 2012 and 2019, according to their clinical presentations. Among all isolates, *emm*4 (30.2%), *emm*3 (18.9%), *emm*1 (12.4%), and *emm*12 (10.1%) were the most prevalent. Among invasive isolates, *emm*1 was the most common genotype identified. Among the isolates from scarlet fever, *emm*4 was most prevalent; however, in the isolates obtained during 2017–2018, *emm*3 was the most common. All *emm* types were included in the proposed 30-valent vaccine. Among all isolates, only 2 (1.2%) isolates demonstrated macrolide resistance and expressed both *erm*A and *erm*B genes.

GAS infection shows differences in spatiotemporal distribution. The distribution of *emm* types among Korean children was similar to that observed in their counterparts in other countries. We found that *emm*1 (27.3%), followed by *emm*12 (18.2%) and *emm*4 (18.2%) were the most prevalent genotypes associated with invasive GAS infections. According to a systematic review, the seven major *emm* types identified (*emm*1, *emm*28, *emm*89, *emm*3, *emm*12, *emm*4, and *emm*6) accounted for approximately 50–70% of the total isolates in countries from Europe and North America (US and Canada) during 2000–2017.[18] Studies have shown *emm*1 to be the most prevalent genotype in Australia, Ireland, and North America.[18–21] However, in Northern European countries such as Denmark, Finland, Norway, and Sweden, *emm*28, *emm*89, and *emm*77 types were identified from isolates associated with invasive infections.[18] In this study, *emm*11 was detected only in a case of invasive infection. In contrast, *emm*11 was reported to have emerged as a prevalent type in both child and adult invasive infections in southern Taiwan.[22] It has also been reported to be related with outbreaks in France and the United States.[23,24]

The distribution of *emm* types among children with scarlet fever in Korea differed from that reported in children from other countries. In China, Hong Kong, and Australia, *emm*12 has been reported as the most prevalent type among affected children.[25–27] Interestingly, the serotype distribution in this study was similar to that reported from England.[28,29] In England, *emm*4 was previously the prevalent serotype; however, *emm*3 has been found to be the most common genotype associated with scarlet fever currently. In this study, *emm*4 (33%) and *emm*3 (25.2%) were

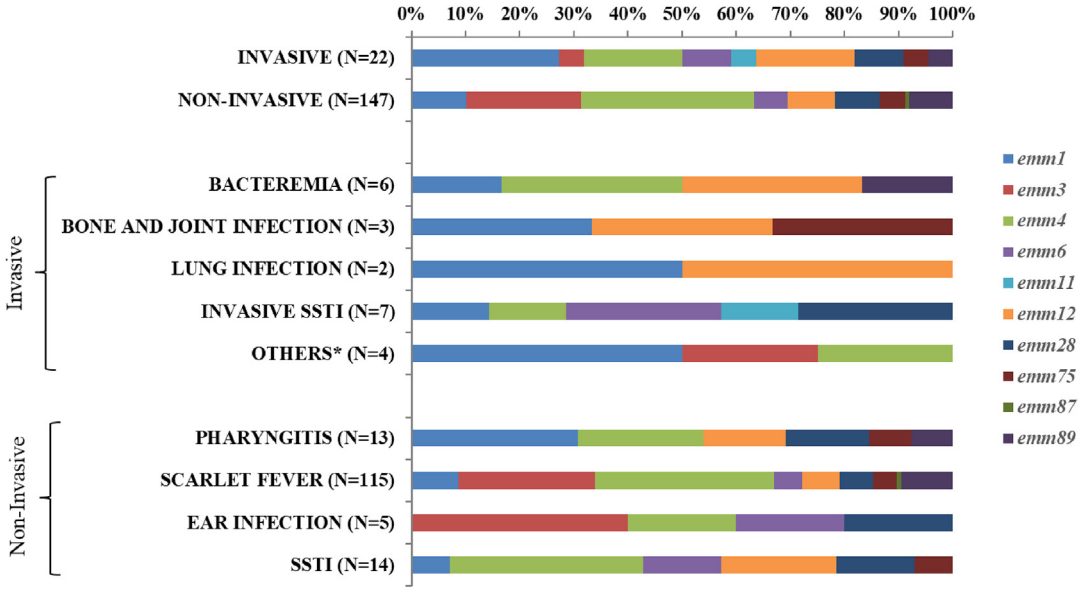

Figure 2. Distribution of *emm* types according to the clinical presentation among children in Korea. *Others include streptococcal toxic shock syndrome (n = 1, *emm*1), deep neck infection (n = 1, *emm*1), CNS infection (n = 1, *emm*3), and infective endocarditis (n = 1, *emm*4). (SSTI: skin and soft tissue infection; CNS: central nervous system).

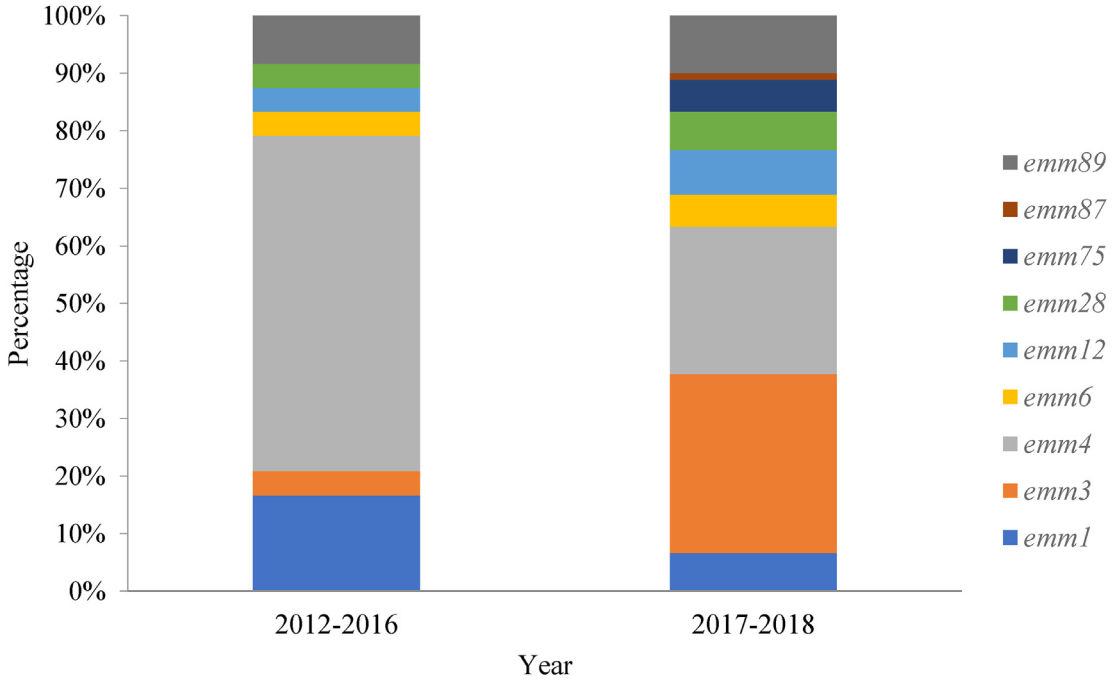

Figure 3. Distribution of *emm* genotypes in scarlet fever isolates, 2012–2016 and 2017–2018.

the most frequently identified M-protein genotypes and *emm*3 was the most common during 2017–2018. Previous studies conducted in South Korea from 1991 to 2012,[30] and from 2008 to 2015,[31] showed that the *emm*4 genotype demonstrated the highest prevalence in isolates of patients with scarlet fever. Continuous surveillance is needed to assess whether the findings in this study reflect a transient increase in *emm*3 or a replacement in the major strain among children with scarlet fever in Korea.

The incidence of scarlet fever varies temporally, and recent epidemiological reports suggest that it has been on the rise. In Singapore, scarlet fever has shown a high incidence rate since an upsurge in 2011.[32] In England, its incidence has been rising since 2014,[28,29] and an increase in its incidence was reported in 2017 in Germany.[33] Similarly, in Korea, a recent increase in cases was observed, with the incidence rates higher in 2017 and 2018, compared to that previously measured over any other period.[34] In this study,

scarlet fever was one of the most common clinical presentations among the affected patients and its incidence has remained high since 2017.

Penicillin is the drug of choice for treating GAS infections in children. To date, there have been no reports of GAS strains showing penicillin resistance. In affected pediatric patients with penicillin allergy, macrolides are a well-known treatment alternative. The GAS resistance rate for erythromycin varies geographically, from 2.5 to 22.4% across countries including Senegal, Greece, France, and Germany[35–38]; in addition, it has demonstrated significant variability over time. In Korea, the erythromycin resistance rate decreased from 51% (2003) to 4.6% (2010)[37,39]; it was 1.2% in this study. Studies that analyzed the *emm* types of erythromycin resistant GAS isolates identified *emm*12 in Greece and Japan,[40,41] *emm*4 in Germany,[33] and *emm*28 in France and Korea[36] as the prevalent genotypes. In this study, *emm*12 and *emm*28 genotypes were found in erythromycin-resistant strains.

This study determined that the proposed 30-valent streptococcal vaccine covered all relevant *emm* types of concern identified in the GAS isolates among children in Korea.[17] With the discovery of newer pathological *emm* types and that of the associated variable severity in clinical presentations, continued epidemiological monitoring of GAS infections in the community is indicated.

This study has certain limitations. Scarlet fever is the only mandatorily reportable GAS infection in Korea; therefore, the isolates collected from participating hospitals were mostly from patients with scarlet fever with a smaller number collected from those with other GAS infections. Regardless, the findings of this multicenter study are relevant, because it included isolates collected from institutions across the country.

In this study, the distribution of *emm* type genes and antimicrobial susceptibility in GAS strains isolated from Korean children between 2012 and 2019 were analyzed according to their clinical presentations. GAS is one of the most common bacterial infections in children and changes in incidence are closely related to the changes in molecular epidemiology. Continuous surveillance of the *emm* type and antimicrobial resistance are important in understanding local and global changes in epidemiology and for formulating management strategies.

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Contributions

Lee H and Cho YN analyzed the data and drafted the initial manuscript. Lee H, Choi EH conceptualized, designed the study and reviewed the manuscript. Park SE, Cho EY, Cho HK, Park J, Kan HM and Yun KW collected the data and critically reviewed the manuscript for important intellectual content. All authors approved the final manuscript as submitted.

Ethical approval

The study was carried out in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of the Seoul National University Bundang Hospital (B-2102-664-302). Written informed consent was waived in this study.

Declaration of competing interest

The authors have no conflict of interests.

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References

1. Zühlke LJ, Beaton A, Engel ME, Hugo-Hamman CT, Karthikeyan G, Katzenellenbogen JM, et al. Group A streptococcus, acute rheumatic fever and rheumatic heart disease: epidemiology and clinical considerations. *Curr Treat Options Cardiovasc Med* 2017;**19**(2):15. https://doi.org/10.1007/s11936-017-0513-y.
2. Walker MJ, Barnett TC, McArthur JD, Cole JN, Gillen CM, Henningham A, et al. Disease manifestations and pathogenic mechanisms of group A Streptococcus. *Clin Microbiol Rev* 2014;**27**(2):264–301. https://doi.org/10.1128/CMR.00101-13.
3. Cole JN, Barnett TC, Nizet V, Walker MJ. Molecular insight into invasive group A streptococcal disease. *Nat Rev Microbiol* 2011;**9**(10):724–36. https://doi.org/10.1038/nrmicro2648.
4. Metzgar D, Zampolli A. The M protein of group A Streptococcus is a key virulence factor and a clinically relevant strain identification marker. *Virulence* 2011;**2**(5):402–12. https://doi.org/10.4161/viru.2.5.16342.
5. Frick IM, Schmidtchen A, Sjöbring U. Interactions between M proteins of Streptococcus pyogenes and glycosaminoglycans promote bacterial adhesion to host cells. *Eur J Biochem* 2003;**270**(10):2303–11. https://doi.org/10.1046/j.1432-1033.2003.03600.x.
6. Johnsson E, Berggård K, Kotarsky H, Hellwage J, Zipfel PF, Sjöbring U, et al. Role of the hypervariable region in streptococcal M proteins: binding of a human complement inhibitor. *J Immunol* 1998;**161**(9):4894–901.
7. Sanderson-Smith M, De Oliveira DM, Guglielmini J, McMillan DJ, Vu T, Holien JK, et al., M Protein Study Group. A systematic and functional classification of Streptococcus pyogenes that serves as a new tool for molecular typing and vaccine development. *J Infect Dis* 2014;**210**(8):1325–38. https://doi.org/10.1093/infdis/jiu260.
8. Shulman ST, Tanz RR, Dale JB, Steer AC, Smeesters PR. Added value of the *emm*-cluster typing system to analyze group A Streptococcus epidemiology in high-income settings. *Clin Infect Dis* 2014;**59**(11):1651–2. https://doi.org/10.1093/cid/ciu649.
9. Spellerberg B, Brandt C. Laboratory Diagnosis of Streptococcus pyogenes (group A streptococci) 2016. In: Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes : basic biology to clinical manifestations [internet]*. Oklahoma City (OK): University of Oklahoma Health Sciences Center; 2016. Available from: https://www.ncbi.nlm.nih.gov/books/NBK343617/.

10. Passàli D, Lauriello M, Passàli GC, Passàli FM, Bellussi L. Group A streptococcus and its antibiotic resistance. *Acta Otorhinolaryngol Ital* 2007;**27**(1):27—32.
11. Seppälä H, Skurnik M, Soini H, Roberts MC, Huovinen P. A novel erythromycin resistance methylase gene (*erm*TR) in Streptococcus pyogenes. *Antimicrob Agents Chemother* 1998;**42**(2): 257—62. https://doi.org/10.1128/AAC.42.2.257.
12. Pankuch GA, Jueneman SA, Davies TA, Jacobs MR, Appelbaum PC. In vitro selection of resistance to four beta-lactams and azithromycin in Streptococcus pneumoniae. *Antimicrob Agents Chemother* 1998;**42**(11):2914—8. https://doi.org/10.1128/AAC.42.11.2914.
13. Jensen LB, Frimodt-Møller N, Aarestrup FM. Presence of *erm* gene classes in gram-positive bacteria of animal and human origin in Denmark. *FEMS Microbiol Lett* 1999;**170**(1):151—8. https://doi.org/10.1111/j.1574-6968.1999.tb13368.x.
14. Fyfe C, Grossman TH, Kerstein K, Sutcliffe J. Resistance to macrolide antibiotics in public health pathogens. *Cold Spring Harb Perspect Med* 2016;**6**(10):a025395. https://doi.org/10.1101/cshperspect.a025395.
15. Clancy J, Petitpas J, Dib-Hajj F, Yuan W, Cronan M, Kamath AV, et al. Molecular cloning and functional analysis of a novel macrolide-resistance determinant, *mef*A, from Streptococcus pyogenes. *Mol Microbiol* 1996;**22**(5):867—79. https://doi.org/10.1046/j.1365-2958.1996.01521.x.
16. Sutcliffe J, Tait-Kamradt A, Wondrack L. Streptococcus pneumoniae and Streptococcus pyogenes resistant to macrolides but sensitive to clindamycin: a common resistance pattern mediated by an efflux system. *Antimicrob Agents Chemother* 1996;**40**(8):1817—24. https://doi.org/10.1128/AAC.40.8.1817.
17. Dale JB, Penfound TA, Chiang EY, Walton WJ. New 30-valent M protein-based vaccine evokes cross-opsonic antibodies against non-vaccine serotypes of group A streptococci. *Vaccine* 2011;**29**(46):8175—8. https://doi.org/10.1016/j.vaccine.2011.09.005.
18. Gherardi G, Vitali LA, Creti R. Prevalent *emm* types among invasive GAS in Europe and North America since year 2000. *Front Public Health* 2018;**6**:59. https://doi.org/10.3389/fpubh.2018.00059.
19. Oliver J, Thielemans E, McMinn A, Baker C, Britton PN, Clark JE, et al. PAEDS investigators. Invasive group A Streptococcus disease in Australian children: 2016 to 2018-a descriptive cohort study. *BMC Publ Health* 2019;**19**(1):1750. https://doi.org/10.1186/s12889-019-8085-2.
20. Meehan M, Murchan S, Gavin PJ, Drew RJ, Cunney R. Epidemiology of an upsurge of invasive group A streptococcal infections in Ireland, 2012—2015. *J Infect* 2018;**77**(3):183—90. https://doi.org/10.1016/j.jinf.2018.05.010.
21. Nelson GE, Pondo T, Toews KA, Farley MM, Lindegren ML, Lynfield R, et al. Epidemiology of invasive group A Streptococcal infections in the United States, 2005—2012. *Clin Infect Dis* 2016;**63**(4):478—86. https://doi.org/10.1093/cid/ciw248.
22. Chiang-Ni C, Wu AB, Liu CC, Chen KT, Lin YS, Chuang WJ, et al. Emergence of uncommon emm types of Streptococcus pyogenes among adult patients in southern Taiwan. *J Microbiol Immunol Infect* 2011 Dec;**44**(6):424—9.
23. Martinaud C, Doloy A, Graffin B, Gaillard T, Poyet R, Mallet S, et al. A family outbreak due to an emm-type 11 multiresistant strain of Streptococcus pyogenes. *Clin Microbiol Infect* 2010 Mar;**16**(3):292—5.
24. Thigpen MC, Thomas DM, Gloss D, Park SY, Khan AJ, Fogelman VL, et al. Nursing home outbreak of invasive group a streptococcal infections caused by 2 distinct strains. *Infect Control Hosp Epidemiol* 2007 Jan;**28**(1):68—74.
25. Chen M, Yao W, Wang X, Li Y, Chen M, Wang G, et al. Outbreak of scarlet fever associated with *emm* 12 type group A Streptococcus in 2011 in Shanghai, China. *Pediatr Infect Dis J* 2012;**31**(9):e158—62. https://doi.org/10.1097/INF.0b013e31825874f3.
26. You Y, Davies MR, Protani M, McIntyre L, Walker MJ, Zhang J. Scarlet fever epidemic in China caused by Streptococcus pyogenes serotype M12: epidemiologic and molecular analysis. *EBioMedicine* 2018;**28**:128—35. https://doi.org/10.1016/j.ebiom.2018.01.010.
27. Walker MJ, Brouwer S, Forde BM, Worthing KA, McIntyre L, Sundac L, et al. Detection of epidemic scarlet fever group A Streptococcus in Australia. *Clin Infect Dis* 2019;**69**(7):1232—4. https://doi.org/10.1093/cid/ciz099.
28. Lamagni T, Guy R, Chand M, Henderson KL, Chalker V, Lewis J, et al. Resurgence of scarlet fever in England, 2014—16: a population-based surveillance study. *Lancet Infect Dis* 2018;**18**(2):180—7. https://doi.org/10.1016/S1473-3099(17)30693-X.
29. Turner CE, Pyzio M, Song B, Lamagni T, Meltzer M, Chow JY, et al. Scarlet fever upsurge in England and molecular-genetic analysis in north-west London, 2014. *Emerg Infect Dis* 2016;**22**(6):1075—8. https://doi.org/10.3201/eid2206.151726.
30. Choi JH, Yang NR, Lee WJ, Lee H, Choi EH, Lee HJ. Distribution of *emm* types among group A Streptococcus isolates from children in Korea. *Diagn Microbiol Infect Dis* 2015;**82**(1):26—31. https://doi.org/10.1016/j.diagmicrobio.2015.01.002.
31. Park DW, Kim SH, Park JW, Kim MJ, Cho SJ, Park HJ, et al. Incidence and characteristics of scarlet fever, South Korea, 2008—2015. *Emerg Infect Dis* 2017;**23**(4):658—61. https://doi.org/10.3201/eid2304.160773.
32. Yung CF, Thoon KC. A 12 year outbreak of scarlet fever in Singapore. *Lancet Infect Dis* 2018;**18**(9):942. https://doi.org/10.1016/S1473-3099(18)30464-X.
33. Brockmann SO, Eichner L, Eichner M. Constantly high incidence of scarlet fever in Germany. *Lancet Infect Dis* 2018;**18**(5): 499—500. https://doi.org/10.1016/S1473-3099(18)30210-X.
34. Kim JH. *Policy development for management of scarlet fever*. Korea Disease Control and Prevention Agency; 2019.
35. Camara M, Dieng A, Boye CS. Antibiotic susceptibility of streptococcus pyogenes isolated from respiratory tract infections in Dakar, Senegal. *Microbiol Insights* 2013;**6**:71—5. https://doi.org/10.4137/MBI.S12996. PMID 24826076.
36. Bingen E, Bidet P, Mihaila-Amrouche L, Doit C, Forcet S, Brahimi N, et al. Emergence of macrolide-resistant Streptococcus pyogenes strains in French children. *Antimicrob Agents Chemother* 2004;**48**(9):3559—62. https://doi.org/10.1128/AAC.48.9.3559-3562.2004.
37. Koh E, Kim S. Decline in erythromycin resistance in group A Streptococci from acute pharyngitis due to changes in the *emm* genotypes rather than restriction of antibiotic use. *Korean J Lab Med* 2010;**30**(5):485—90.
38. Reinert RR, Lütticken R, Bryskier A, Al-Lahham A. Macrolide-resistant Streptococcus pneumoniae and Streptococcus pyogenes in the pediatric population in Germany during 2000—2001. *Antimicrob Agents Chemother* 2003;**47**(2):489—93.
39. Park SJ, Kim S. Antibiotic resistance and its mechanism of group A streptococci in school children of Jinju. *Korean J Clin Microbiol* 2003;**6**:7—11.
40. Michos AG, Bakoula CG, Braoudaki M, Koutouzi FI, Roma ES, Pangalis A, et al. Macrolide resistance in Streptococcus pyogenes: prevalence, resistance determinants, and emm types. *Diagn Microbiol Infect Dis* 2009;**64**(3):295—9. https://doi.org/10.1016/j.diagmicrobio.2009.03.004.
41. Wajima T, Murayama SY, Sunaoshi K, Nakayama E, Sunakawa K, Ubukata K. Distribution of emm type and antibiotic susceptibility of group A streptococci causing invasive and noninvasive disease. *J Med Microbiol* 2008;**57**(11):1383—8.