



## Case report

## Characteristics of mucoid *Streptococcus pyogenes* isolated from two patients with pneumonia in a local community



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## ABSTRACT

*Streptococcus pyogenes* (Group A Streptococcus, GAS) infections can develop into life-threatening disorders. However, the occurrence of some GAS pneumonia cases is relatively rare in a local community. We report here characteristics of mucoid GAS isolates obtained from the sputum of two patients with pneumonia in a local community. Although case-patients did not have contact with each other, case-patient 1's child and case-patient 2's grandchild attended the same kindergarten where a GAS pharyngitis epidemic had occurred. We conducted phenotypic and genotypic analyses with the GAS isolates from sputum of both patients, to examine (1) colony appearance between the isolates, (2) numerical profile based on API-20 Strep system, (3) similarity to the type strain using 16S rRNA sequencing, (4) *emm* type (subtype) and *emm* full-length sequence, (5) sequence type, (6) *sic* allele, (7) antimicrobial susceptibility result and the resistance determinant, (8) genome profile following a random amplified polymorphic DNA fragments, and (9) pattern of digested DNA fragments by pulse-field gel electrophoresis. These phenotypic and genotypic analyses revealed similar matching between the isolates from both cases. Our findings suggest that when clinicians examine adult patients having infection with the mucoid GAS, they should confirm whether anyone within the same household also developed the infection and need to investigate epidemic situations in local communities, including kindergartens and elementary schools.

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## Introduction

*Streptococcus pyogenes* (Group A Streptococcus, GAS) infections can develop into life-threatening disorders. GAS can be spread from person to person via droplets or through contact with infected wounds. Some reports describe nosocomial transmission and spread among family members and nursing homes residents [1,2]. But GAS pneumonia was relatively unusual in those settings.

Herein, we report our clinical experience with two temporally proximal cases of pneumonia from whose sputum mucoid GAS were isolated and phenotypic and genotypic analyses conducted

with the GAS isolates to assess the potential epidemiological relatedness.

## Case 1

A 40-year-old woman presented to the emergency department with fever, cough, diarrhea and vomiting 4 days prior to the admission. The patient did not have a significant past medical history. Her children also had sore throat and fever 2 days before the onset of her symptoms. On examination, her temperature was 39.6 °C, blood pressure was 90/60 mmHg, pulse rate was 116 beats/min, respiratory rate was 30 breaths/min, and oxygen saturation was 95% (40% fraction of inspired oxygen, 9 L/min). Laboratory tests showed leukocytosis (22,800/μL), elevated C-reactive protein level (30.8 mg/dL), renal functional damage (creatinine: 1.93 mg/dL), and disseminated intravascular coagulation, suggesting a toxic shock syndrome. A chest X-ray and computed tomography

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revealed infiltration with air bronchogram in the right lower lobe and bilateral pleural effusions. The patient was diagnosed with severe pneumonia accompanied by septic shock and treated with an intravenous antimicrobial combination of levofloxacin (LVFX; 500 mg every 24 h) and ceftriaxone (CTRX; 2 g every 12 h), in addition to recombinant human thrombomodulin. When the blood and sputum cultures grew *S. pyogenes*, this regimen was simplified to penicillin G (3 million units every 4 h) and clindamycin (600 mg every 8 h). Since renal function was exacerbated and pleural effusion increased, she also required hemodialysis and chest tube placement. Her condition was subsequently complicated by a drug-induced fever, skin eruption, and pneumonia. Therefore, the combination changed just to CTRX (2 g every 24 h) on day 14 and then to LVFX (500 mg every 24 h) on day 20. The patient completed a course for 24 days and was then discharged.

## Case 2

An 84-year-old woman was admitted after experiencing a cough and dyspnea on exertion for 3 days. Although she had mycosis fungoides, she was not taking medication for this disease. Her grandchild had a sore throat several days before the onset of her symptoms. Although the patient lived away from the grandchild, she had contact with the grandchild preclinically. The patient was taking medications for vertebral canal stenosis. In addition to those, clarithromycin and carbocisteine were prescribed for her respiratory symptoms by her primary care physician on the day before admission. Her temperature was 37.3 °C, blood pressure was 171/92 mmHg, pulse rate was 94 beats/

min, respiratory rate was 28 breaths/min, and oxygen saturation was 90% at ambient air. Laboratory tests showed leukocytosis (15,600/ $\mu$ L), an elevated C-reactive protein level (17.3 mg/dL), and slightly elevated aminotransferases (aspartate transaminase: 45 U/L; alanine transaminase: 38 U/L). A chest X-ray revealed pulmonary infiltrate with air bronchogram in the right lower lobe. The patient was diagnosed with community-acquired pneumonia and treated with intravenous ampicillin/sulbactam (ABPC/SBT; 3 g every 6 h). A sputum culture yielded *S. pyogenes*, but the blood culture did not. She improved quickly and was then treated with ABPC/SBT for 7 days.

These two cases occurred 2 weeks apart, and case-patient 1's child and case-patient 2's grandchild had experienced self-limited pharyngitis. Although the case-patients did not contact each other, case-patient 1's child and case-patient 2's grandchild attended the same kindergarten. Other children attending this kindergarten also experienced pharyngitis within that time frame, suggesting an epidemiological relationship of this GAS strain.

## Microbiological analyses

To investigate the potential homology between the GAS isolates (M1 and M2) from sputum cultures, we examined phenotypic and genotypic characteristics. However, throat swabs specimens could not be obtained from the remaining family members. Phenotypic analyses examined colonial morphology on sheep blood agar plate, the percent identification by numerical profile using the API-20 Strep system (SYSMEX bioMérieux Co., Ltd., Tokyo, Japan), and antimicrobial susceptibility data using the broth microdilution

**Table 1**

Phenotypic and genotypic characteristics and antimicrobial susceptibility results of *Streptococcus pyogenes* isolates from two patients.

	M1	M2
Phenotypic and genotypic parameters		
Clinical specimen	Sputum	Sputum
Gross appearance of colonies on sheep blood agar plate	Mucoid	Mucoid
Numerical profile using the API-20 Strep system (% identification)	0161417 (99.9)	0161417 (99.9)
Similarity (%) to <i>S. pyogenes</i> type strain <sup>a</sup> using 16S rRNA sequencing (sequencing size, bp)	100 (1418)	100 (1418)
<i>emm</i> type (subtype)	1(0.0)	1(0.0)
<i>emm</i> full-length (sequencing size, bp)	Identical to that of <i>S. pyogenes</i> MGAS5005 strain (1093)	Identical to that of <i>S. pyogenes</i> MGAS5005 strain (1093)
Sequence type (allelic profile: <i>gki-gtr-murl-mutS-recP-xpt-yqil</i> )	28 (4–3–4–4–4–2–4)	28 (4–3–4–4–4–2–4)
Streptococcal inhibitor of complement ( <i>Sic</i> ) allele No. (sequencing size, bp)	<i>Sic1.32</i> (931)	<i>Sic1.32</i> (931)
Antimicrobial agent resistance class <sup>b</sup>	macrolide	macrolide
Macrolide resistance determinant	<i>mef(A)</i>	<i>mef(A)</i>
Antimicrobial agents		
	Minimum inhibitory concentration	
Penicillin G	≤0.03	≤0.03
Ampicillin	≤0.06	≤0.06
Cefepime	≤0.5	≤0.5
Cefotaxime	≤0.12	≤0.12
Ceftriaxone	≤0.12	≤0.12
Meropenem	≤0.12	≤0.12
Vancomycin	0.5	0.5
Erythromycin	>2	>2
Azithromycin	>4	>4
Clindamycin	≤0.12	≤0.12
Minocycline	1	1
Levofloxacin	1	1
Chloramphenicol	≤4	≤4

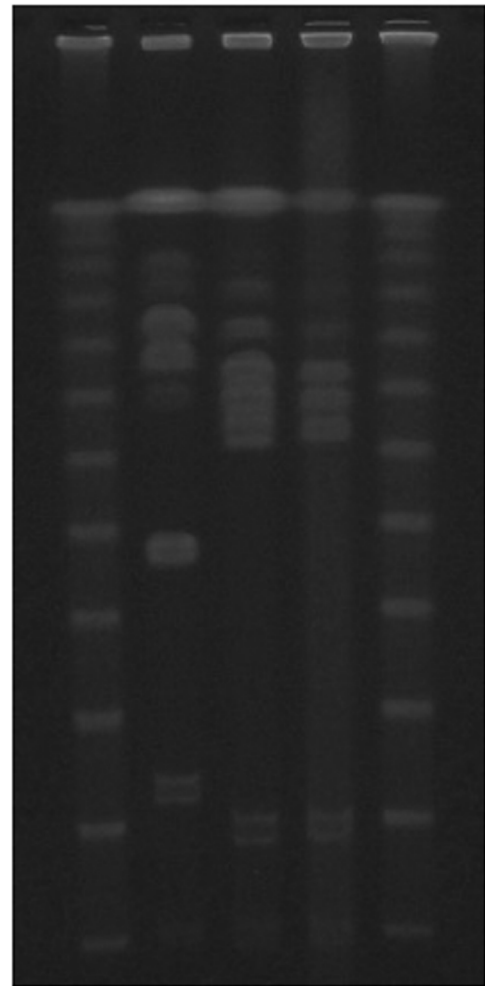
<sup>a</sup> *S. pyogenes* JCM 5674(T).

<sup>b</sup> Resistance to antimicrobials was determined by the broth microdilution method according to the Clinical and Laboratory Standards Institute document M100-S22.

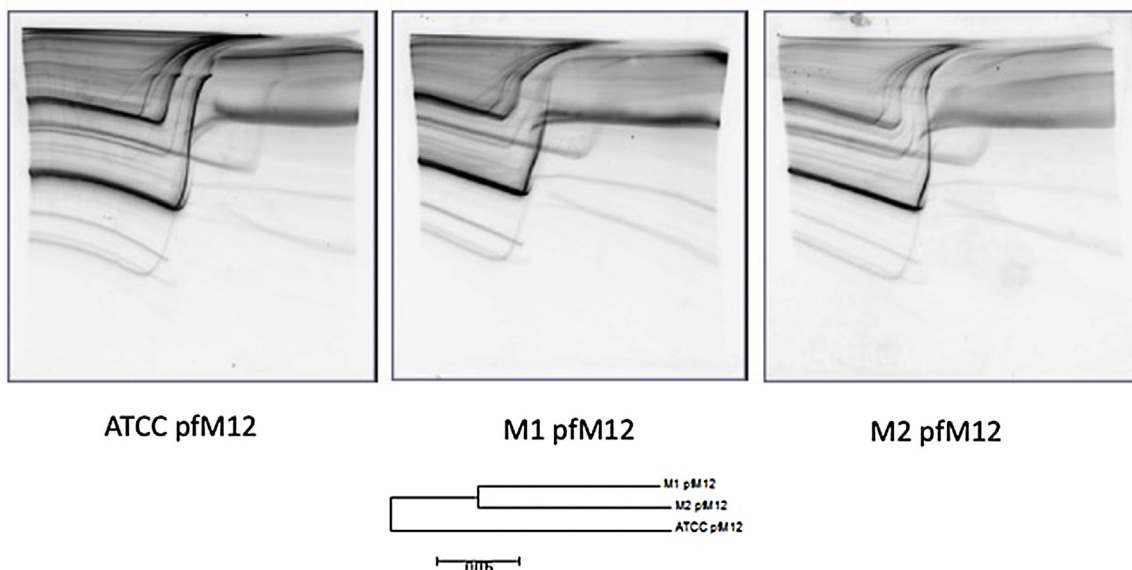
method [3]. Genomic analyses compared the similarity (in%) of the M1/M2 and the type strain using 16S rRNA gene sequencing [4], determined the *emm* type (subtype), with the full-length sequences [5], sequence type (ST) [6], *sic* allele [7], and macro-lide/lincosamide (ML) resistance determinants including *erm(A)*, *erm(B)*, and *mef(A)* [8]. Briefly, all *emm* typing (sub-typing) was based on the Centers for Disease Control and Prevention database (<http://www2a.cdc.gov/ncidod/biotech/strepblast.asp>), and the full-length sequencing was done with the same polymerase chain reaction (PCR) primers. Multilocus sequence typing (MLST) to obtain ST was performed by sequencing 7 housekeeping genes (*gki*, *gtr*, *murI*, *mutS*, *recP*, *xpt*, and *yqjL*) according to the GAS pubMLST website (<http://pubmlst.org/spyogenes/>). The *sic* gene was amplified with primer pair (SIC.1/SIC.2), and we did the sequencing with two sequencing primers SIC.3/SIC.4. The *sic* allele number was determined and assigned by comparison to the reference allele. The ML resistant gene was amplified by PCR, and was confirmed by the corresponding amplicon size on agarose gel electrophoresis. We also performed genome profiling (GP) method based on random PCR following a random amplified polymorphic DNA fragments using a pFM12 primer with three GAS strains (ATCC 12344(T), M1, and M2) to clarify the genetic relatedness between M1 and M2 [9]. Briefly, we did (1) reproducible sampling of DNA fragments from the original genomic DNA by random PCR, (2) acquisition of the sequence-derived information without sequencing by using micro-temperature gradient gel electrophoresis, into which random PCR products can be applied without purification of DNA fragments, and (3) normalization of the acquired data using internal references. Pulse-field gel electrophoresis (PFGE) of *SfiI*-digested DNA fragments with the same GAS strains [10] was also carried out to confirm difference in the patterns of DNA fragments from the ATCC strain and clinical isolates.

These phenotypic and genotypic characteristics including antimicrobial susceptibility results are shown in Table 1. Both the GP-induced clustering image and the PFGE-induced patterns of DNA fragments indicated similar, but not identical, clonality between M1 and M2, but these strains were different from ATCC12344 (Figs. 1 and 2). This similarity, but not identical, clonality appears to be due to potential pathogen passage through the local community.

**Marker ATCC M1 M2 Marker**



**Fig. 2.** Pulse-field gel electrophoresis of *SfiI*-digested DNA fragments from the same GAS strains (ATCC 12344(T), M1, and M2).



**Fig. 1.** Genome profiling based on random polymerase chain reaction using a pFM12 primer with three GAS strains (ATCC 12344(T), M1, and M2).

## Discussion

Risk of GAS transmission from index cases to contacts depends on the duration of exposure and the distance from the index case [11]. According to data from the Tokyo Metropolitan Infectious Disease Surveillance Center ([survey.tokyo-eiken.go.jp/epidinfo/zensu10bchart.do](http://survey.tokyo-eiken.go.jp/epidinfo/zensu10bchart.do)), the period in which the two patients had GAS pneumonia did not occur during a GAS infection peak. GAS isolates from the both patients most likely transmitted from the classmates in the kindergarten who had been infected with pharyngitis. Therefore, clinicians should also take a history concerning epidemic information in the local community.

ST28, the same ST type as our isolates, is the most dominant type in the MLST GAS database (<http://pubmlst.org/spyogenes/>) and is also a major ST type in Japan in an epidemiological study of erythromycin-resistant GAS [6]. In Japan, the incidence of invasive GAS infections doubled from 2010 to 2012. The predominant genotype was *emm1* [12], the same as the isolates in this study. A study using clinical isolates and mouse bone-marrow-derived macrophages showed that the level of inflammatory mediators produced was dependent on the *emm* type and *emm1* type isolates are able to induce the highest levels of pro-inflammatory cytokines [13]. However, individuals infected with the same clone can develop different clinical manifestations [14].

In our cases, case-patient 2 did not develop streptococcal toxic shock syndrome, while case-patient 1 did, suggesting differences in the disease manifestations due to *emm1*/ST28 or preexisting superantigen antibody in the second patient. The roles of host cellular mediators during GAS infection are poorly understood [15]. Nevertheless, recent studies have shown that host leukocyte receptor binding to GAS-derived products mediates the release of inflammatory mediators associated with severe GAS disease. Kotb et al. suggest that the variation in class II alleles/haplotypes can influence invasive GAS infection outcomes indicating that host factors may play an important role in the development of invasive GAS infections [14]. We additionally considered that the difference of disease course between both patients may result from the frequency of contact with their child or grandchild and the length from initial symptoms to visiting medical attention.

The *sic* gene encodes an extracellular protein known as streptococcal inhibitor of complement secreted by serotype M1 and M57 GAS. Changes in *sic* in during epidemics within a population have been reported previously, but these events may require prolonged carriage [7]. We suggest that changes in *sic* between both patients were not identified, as both patients were temporally proximal cases of GAS pneumonia.

The GAS strains isolated from our cases were mucoid. The mucoid phenotype is due to increased production of capsular polysaccharide including hyaluronate. A report investigating a mucoid strain outbreak in Spain showed that invasive disease was more often due to mucoid isolates than non-mucoid isolates (3% vs. 0.21%,  $p = 0.0001$ ) [16]. Studies have emphasized the importance of mucoid isolate detection in clinical practice. The isolation of mucoid GAS colonies suggests an epidemic in the local community and may help to prevent an outbreak of dangerous GAS infection in local hospitals.

In summary, based on phenotypic and genotypic analyses, we showed similar clonality of mucoid GAS isolates from two temporally proximal cases of pneumonia. When examining adult patients having infection with the mucoid GAS, clinicians should confirm whether anyone within the same household also developed the infection. If any relatives have been affected, clinicians also need to investigate epidemic situations in the relatives' communities, including kindergartens and elementary schools.

## Conflict of interest statement

None to declare.

## Informed consent

Written informed consent was obtained from both patients (case 1 and 2) for publication of this article. A copy of the written consent is available for review by Editor-in-Chief of this journal on request.

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