



Case report

Repetitive cellulitis caused by *Streptococcus agalactiae* isolates with different genotypic and phenotypic features in a patient having upper extremity with lymphedema after mastectomy and axillary lymph node dissection



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ABSTRACT

Previously reported cases of recurrent cellulitis/erysipelas affecting chronically lymphedematous skin regions have been demonstrated to be due to *Streptococcus agalactiae* isolates with closely related genetic background which may be suggestive of relapse rather than reinfection. Herein, we report the occurrence of three episodes of repetitive cellulitis caused by *S. agalactiae* strains with different genotypic and phenotypic characteristics, including different antimicrobial susceptibility patterns (tetracycline, macrolide/lincosamide, and fluoroquinolone classes), in the left upper extremity of a patient with lymphedema, following left mastectomy and axillary lymph node dissection. The genotypic and phenotypic characteristics of the three isolates were confirmed based on the random amplified polymorphic DNA patterns, DNA profiles of virulence factors (*bca-rib-bac-lmb-cylE*), data on biofilm formation and cell invasion, antimicrobial susceptibility testing results, antimicrobial resistance (AMR) genotypes, and amino acid mutations associated with AMR. These results revealed that reinfection with *S. agalactiae*, rather than recurrence, occurred during the three episodes. In conclusion, microbiologic studies such as blood cultures or tissue cultures are certainly helpful in the management of recurrent infections or invasive infections such as bacteremia in order to better target antimicrobial therapy, regardless of the data previously presented.

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Introduction

Streptococcus agalactiae is the species designated for streptococci belonging to Lancefield carbohydrate group B. In general, group B *Streptococcus* (GBS) is facultative, beta-hemolytic, and Gram-positive coccus that can divide in pairs and chains and can grow readily on variety of growth mediums. This GBS is a frequent colonizer in the lower gastrointestinal and female urogenital tracts. The incidence of invasive GBS disease in neonates has decreased since the introduction of intrapartum prophylactic

antibiotic therapy for pregnant women found to be colonized with GBS, while some studies have shown an increase in the incidence of invasive infections in adults [1,2]. Invasive GBS infection among adults is frequently severe and associated with substantial morbidity and mortality. The most frequent clinical syndromes are primary bacteremia without evident foci, skin and soft tissue infections (SSTIs) including cellulitis, abscess, infected decubitus ulcer, osteomyelitis, septic arthritis, and wound infection. SSTIs are the most common sites of focal GBS infection in adults accounting for more than one-third of infections in some reports [2,3]. Approximate 4% of non-pregnant adults surviving from an episode of GBS bacteremia experience the second episode for at least 1-year follow-up period [4]. However, little is known regarding both host and microbiological factors that may predispose adults to developing repetitive GBS infections. When chronic lymphedema is present in clinical setting, recurrence/relapse is more common [5]. In fact, recurring cellulitis is a known complication in breast cancer patients who suffer post-operative upper extremity

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lymphedema following axillary lymph node dissection when undergoing an operation [6].

Herein, we report a case presenting with three episodes of recurrent cellulitis in the left upper extremity, following left mastectomy and axillary lymph node dissection at her operation. GBS strains harboring different genotypic and phenotypic characteristics (including different antimicrobial susceptibility patterns) were the causative agents leading to the three episodes.

Case

A 56-year-old woman with medical complication of atopic dermatitis, left breast cancer, and cellulitis in the left upper extremity (two episodes) presented with rigor and fever. It was unclear whether the cellulitis was caused by GBS: which antibiotics were used to treat these two episodes: and whether she was previously exposed to antibiotics other than the ones to treat these episodes. She denied any contact with animals,

including pets. At 41 years of age, she underwent total left mastectomy and left axillary lymph node dissection. Thereafter, she had received hormone therapy for breast cancer for 7 years and had been in remission for 8 years. On admission, the patient presented with a temperature of 39.1 °C, blood pressure of 130/80 mmHg, respiratory rate of 20 breaths/min, subcutaneous oxygen saturation of 98 % (equivalent to ambient concentration), and heart rate of 98 bpm. Physical examination revealed remarkable swelling and redness of the left arm. The leukocyte cell count and C-reactive protein (CRP) concentration were 12,600 cells/mm³ and 0.30 mg/dL, respectively. The patient was diagnosed with cellulitis and intravenous administration of ceftriaxone was initiated at 2 g/day (administered as two 1-g doses). Duplicate blood cultures collected on admission grew GBS / *S. agalactiae*. The urine culture on admission also grew GBS, along with negative Gram-staining result and no evidence of phagocytosis. She developed asymptomatic situations, not suggestive of urinary tract infection. The urinalysis on admission showed negative nitrous acid reaction and no

Table 1
Phenotypic and genotypic features of *Streptococcus agalactiae* isolates from a case of repetitive cellulitis in the left upper extremity with lymphedema after the left mastectomy.

Strain	GB25	GB88	GB93
Isolation date (yr/mon/day)	2017/Jul/26	2018/Feb/10	2018/Dec/22
Clinical specimen	Blood	Blood	Blood
Gross appearance of colonies on a sheep blood agar plate	Non-mucoid, beta-hemolytic small gray-white-colored smooth colonies	Non-mucoid, beta-hemolytic small gray-white-colored smooth colonies	Non-mucoid, beta-hemolytic small gray-white-colored smooth colonies
Carbohydrate group (Lancefield antigen)	Group B	Group B	Group B
Identification score value by MALDI-TOF MS	1.99	2.39	2.00
Similarity (%) to <i>S. agalactiae</i> type strain (ATCC 13813) using 16S rRNA sequencing (sequencing size, bp)	99.86 (706)	100 (711)	99.86 (710)
<i>S. agalactiae</i> -specific gene, <i>dltS</i> encoding histidine kinase (sensor protein in the membrane)	Positive	Positive	Positive
Capsular genotype	Ib	V	Ib
Sequence type (allelic profile: <i>adhP-pheS-atr-glnA-sdhA-glck-tkt</i>)	703 (9-1-4-1-3-57-2)	1 (1-1-2-1-1-2-2)	10 (9-1-4-1-3-3-2)
Random amplified polymorphic DNA pattern from each isolate ^a	Different from each other	Different from each other	Different from each other
PCR-based DNA profile of virulence factors	<i>bca-bac-lmb-cylE</i>	<i>bca-rib-lmb-cylE</i>	<i>bca-rib-bac-lmb-cylE</i>
Biofilm formation (absorbance, mean ± SD of 5 wells) ^a	0.04 ± 0.008 ^c	0.01 ± 0.004 ^c	0.07 ± 0.010 ^c
Cell invasion ability (number of invaded <i>S. agalactiae</i> /100 cells, mean ± SD of 4 wells) ^a	0.12 ± 0.01 ^d	0.10 ± 0.02 ^d	0.17 ± 0.03 ^d
Resistant antimicrobial agent class ^b	Fluoroquinolone alone	Tetracycline alone	Tetracycline/macrolide/lincosamide/fluoroquinolone
AMR genotype and amino acid mutations associated with AMR	Ser79Phe in <i>parC</i> & Ser81Leu in <i>gyrA</i>	<i>tet(M)</i>	<i>tet(O)</i> , <i>erm(B)</i> , Ser79Phe in <i>parC</i> & Ser81Leu in <i>gyrA</i>
Antimicrobial agents	MIC (μg/mL)	MIC (μg/mL)	MIC (μg/mL)
Penicillin G	≤ 0.03	≤ 0.03	≤ 0.03
Ampicillin	≤ 0.06	≤ 0.06	≤ 0.06
Amoxicillin/clavulanic acid	≤ 0.25	≤ 0.25	≤ 0.25
Cefotiam	≤ 0.5	≤ 0.5	≤ 0.5
Cefazolin	0.25	0.25	0.5
Ceftriaxone	≤ 0.12	≤ 0.12	≤ 0.12
Cefepime	≤ 0.06	≤ 0.06	0.12
Cefmetazole	0.12	> 2	1
Cefditoren pivoxil	≤ 0.25	≤ 0.25	≤ 0.25
Meropenem	0.03	0.03	0.03
Azithromycin	≤ 0.12	1	> 4
Clindamycin	0.12	0.12	> 0.5
Minocycline	≤ 0.12	> 4	> 4
Chloramphenicol	> 2	2	2
Vancomycin	0.5	0.5	0.5
Levofloxacin	> 4 ^e	1	> 4 ^e
Sulfamethoxazole-trimethoprim	≤ 5	≤ 5	≤ 5

MALDI-TOF MS, matrix-assisted laser desorption ionization-time of flight mass spectrometry; AMR, antimicrobial resistance; MIC, minimum inhibitory concentration.

^a The type strain (ATCC 13813) of *S. agalactiae* was applied as quality controls for phenotypic and genotypic analyses.

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

^c $p < 0.01$ GB25 vs. GB88, GB88 vs. GB93, and GB25 vs. GB93 using Welch's *t*-test following the F-test.

^d $p < 0.05$ GB25 vs. GB93, $p < 0.01$ GB88 vs. GB93 using Welch's *t*-test following the F-test.

^e The MIC values (μg/mL) by Etest using norfloxacin/moxifloxacin against GB25 and GB93 revealed 256/3 and 256/8, respectively.

evidence of pyuria. These findings indicated the possibility of GBS colonization in the genitourinary tracts. Ceftriaxone treatment was replaced with intravenous administration of ampicillin at 4 g/day (administered as two 2-g doses). The patient was administered parental antibiotics for 10 days, followed by amoxicillin at 1.5 g/day (administered as three 0.5-g doses) for 7 days.

The patient presented with rigor and pain in the upper left extremity 6 months later. On admission, the patient presented with a temperature of 37.4 °C, blood pressure of 150/78 mmHg, respiratory rate of 20 breaths/min, subcutaneous oxygen saturation of 99 % (equivalent to ambient concentration), and heart rate of 120 bpm. Physical examination revealed remarkable swelling and redness of the left arm. The leukocyte cell count and CRP concentration were 22,700 cells/mm³ and 0.30 mg/dL, respectively. The patient was diagnosed with cellulitis and intravenous administration of meropenem was initiated at 2 g/day (administered as two 1-g doses). Duplicate blood cultures collected on admission grew GBS / *S. agalactiae*. Meropenem treatment was replaced with intravenous administration of ampicillin at 8 g/day (administered as four 2-g doses). The patient was administered parental antibiotics for 14 days.

The patients presented with fever and pain in the upper left extremity again 10 months later. On admission, the patient presented with a temperature of 39.4 °C, blood pressure of 118/80 mmHg, respiratory rate of 18 breaths/min, subcutaneous oxygen saturation of 96 % (equivalent to ambient concentration), and heart rate of 96 bpm. Physical examination revealed remarkable swelling and redness of the left arm. The leukocyte cell count and CRP concentration were 16,100 cells/mm³ and 0.30 mg/dL, respectively. The patient was diagnosed with cellulitis and intravenous administration of ampicillin/sulbactam was initiated at 4.5 g/day (administered as three 1.5-g doses). Duplicate blood cultures collected on admission grew GBS / *S. agalactiae*. Ampicillin/sulbactam treatment was replaced with intravenous administration of ampicillin at 8 g/day (administered as four 2-g doses). The patient was administered parental antibiotics for 14

days, followed by amoxicillin/clavulanate at 0.75 g/day (administered as three 0.25-g doses) for 7 days. Treatment was successful; the patient remained well, with no recurrence of bacteremia, during 22 months of follow up.

Microbiological analyses

All blood-origin isolates from the third to the fifth episode of cellulitis were stored at −80 °C until further evaluation. We examined the phenotypic and genotypic characteristics of the GBS isolates (GB25, GB88, and GB93) from the patient's blood (Table 1). The GBS isolates were identified using colonial morphology on sheep blood agar. Identification score values from matrix-assisted laser desorption ionization-time of flight mass spectrometry and antimicrobial susceptibility testing (AST) data using broth microdilution method according to the Clinical and Laboratory Standards Institute document M100-S22 were obtained. Minimum inhibitory concentrations (μg/mL) of norfloxacin and moxifloxacin against GB25 and GB93 were determined using Etest. Both biofilm formation (BF) and cell invasion ability (CIA) were determined for all isolates [7]. Genotypic analyses were performed using 16S rRNA gene sequencing [8] and capsular genotyping with amplification of species-specific gene (*dltS*) [9]. Sequence type was determined using multilocus sequence typing (allelic profile: *adhP*–*pheS*–*atr*–*glnA*–*sdhA*–*glcK*–*tkl*) and the pubMLST website (<https://pubmlst.org/sagalactiae/>) [9]. We analyzed random amplified polymorphic DNA (RAPD) banding patterns using three different primers–H2, P5, and P6 [7], polymerase chain reaction-based DNA profiles of virulence factors (*bca*–*rib*–*bac*–*lmb*–*cylE*) [10–12] and tetracycline-class and macrolide/lincosamide-class resistance determinants [*tet*(M)–*tet*(O)–*tet*(K)–*tet*(L)–*tet*(S) and *erm*(A)–*erm*(B)–*mef*(A)] [8]. Point mutations at quinolone resistance-determining regions of DNA topoisomerase IV and gyrase were examined [13].

We found that the genotypic and phenotypic characteristics, including RAPD banding pattern (Fig. 1), DNA profiles of virulence

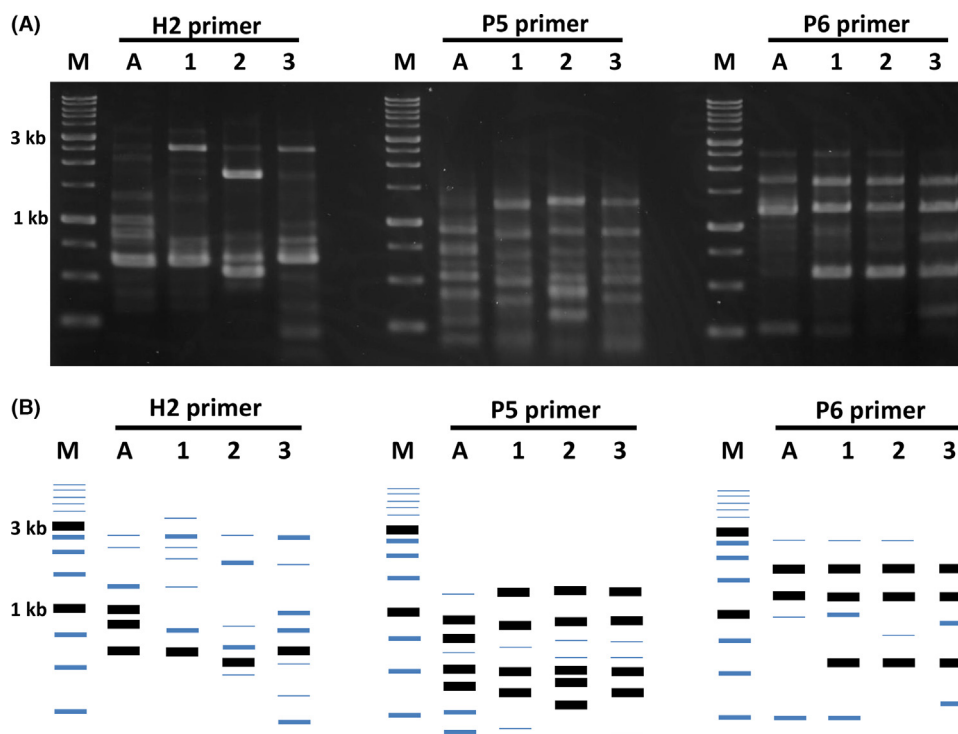


Fig. 1. Random amplified polymorphic DNA (RAPD) analysis of *S. agalactiae* isolates causing repetitive cellulitis (A) and the schematic RAPD banding pattern (B). The primers H2, P5, and P6 were used. M, marker; A, ATCC 13813; 1, GB25; 2, GB88; 3, GB93.

factors, BFs/CIAs data, AST results, and antimicrobial resistance genotypes, were different for all three isolates (Table 1).

Discussion

This pathogen has been isolated from several invasive infections, including sepsis/bacteremia with unknown foci, infectious endocarditis, septic arthritis, meningitis, and others in human neonatal/elderly subjects and companion animals [8,9,14]. Therefore, human and veterinary clinicians should be aware of the potential risk for infectious diseases resulting from this bacterium.

Rodriguez et al. reported clinical and microbiological characteristics regarding cellulitis following lymphedema of the extremities [15]. The prevalence and recurrence rates of cellulitis in 420 patients with lymphedema of the extremities were found to be 12.6 % (53/420) and 56.6 % (30/53), respectively. A total of 131 independent episodes were documented from 43 (81.1 %) lower limbs and 10 (19.9 %) upper limbs. Blood cultures were obtained for 79 (60.3 %) episodes, with 9 (11.4 %) testing positive; GBS was the most isolated strain (5 of 9; 55.5 %). Previously reported cases of recurrent cellulitis/erysipelas affecting chronically lymphedematous skin regions have been demonstrated to be due to *S. agalactiae* isolates with closely related genetic background which may be suggestive of relapse rather than reinfection [16–18]. Vaginal GBS colonization or intracellular persistence of GBS in non-professional phagocytes, fibroblasts, epithelial, and endothelial cells was demonstrated as a potential mechanism in recurrent cellulitis/erysipelas [18,19]. There was also the possibility of GBS colonization in the genitourinary tracts of this patient. However, the first, the second, and the third isolates showed the different genotypic/phenotypic characteristics, suggesting the heterogeneity in strains that were endogenous to this patient and that were exogenously not acquired. Clinicians encountering patients who develop repetitive infections with beta-hemolytic streptococci should be aware of the following possibilities: recurrence due to closely related strains and reinfection by different strains [7].

Prophylactic antibiotic strategy to prevent recurrent cellulitis have been recommended with reservation due to conflicting data [20]. Given the general susceptibility of GBS to beta-lactams, the heterogeneity in strains does not invalidate prophylaxis with beta-lactams (except for the beta-lactam allergy). Additionally, non-pharmacologic risk reduction is prudent regardless of whether different strains or species are responsible for recurrence or reinfection.

Conclusion

In fact, the first, the second, and the third isolates revealed the different AST patterns resistant to fluoroquinolone alone, to tetracycline alone, and to tetracycline/macrolide/lincosamide/fluoroquinolone, respectively. We needed to choose the limited antimicrobials according to the AST result determined when each episode occurred. Therefore, microbiologic studies such as blood cultures or tissue cultures are certainly helpful in the management of recurrent infections or invasive infections such as bacteremia in order to better target antimicrobial therapy, regardless of the data previously presented. On the other hand, one can argue that the general susceptibility of GBS to beta-lactams makes the variable susceptibility to the second line agents less clinically relevant.

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Informed consent

The patient gave his informed consent before this article was written.

Author contribution

Daisuke Taniyama and **Takashi Takahashi** contributed to the report concept and design.

Daisuke Taniyama and **Taketomo Maruki** performed the acquisition of patient's data.

Takahiro Maeda, **Haruno Yoshida**, and **Takashi Takahashi** contributed to the phenotypic/genotypic analyses using the isolates.

Daisuke Taniyama and **Takashi Takahashi** prepared and wrote the manuscript.

Ethical approval

Ethical approval was not required for this study.

Declaration of Competing Interest

The authors have disclosed no relevant financial relationships.

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References

- [1] Henning KJ, Hall EL, Dwyer DM, Billmann L, Schuchat A, Johnson JA, et al. Maryland Emerging Infections Program. Invasive group B streptococcal disease in Maryland nursing home residents. *J Infect Dis* 2001;183:1138–42.
- [2] Schwartz B, Schuchat A, Oxtoby MJ, Cochi SL, Hightower A, Broome CV. Invasive group B streptococcal disease in adults. A population-based study in metropolitan Atlanta. *JAMA* 1991;266:1112–4.
- [3] Farley MM, Harvey RC, Stull T, Smith JD, Schuchat A, Wenger JD, et al. A population-based assessment of invasive disease due to group B Streptococcus in nonpregnant adults. *NEJM* 1993;328:1807–11.
- [4] Harrison LH, Ali A, Dwyer DM, Libonati JP, Reeves MW, Elliott JA, et al. Relapsing invasive group B streptococcal infection in adults. *Ann Intern Med* 1995;123:421–7.
- [5] Cox NH. Oedema as a risk factor for multiple episodes of cellulitis/erysipelas of the lower leg: a series with community follow-up. *Br J Dermatol* 2006;155:947–50.
- [6] Faisal Cheema, Maliha Agloria, Glenn Morris Mills, Runhua Shi, Benjamin Li, et al. Recurring breast and arm cellulitis in patients with early breast cancer. <http://meetinglibrary.asco.org/content/117756-132> (abstract 31.e20600) [Accessed 14 December 2019].
- [7] Fujita T, Yoshida H, Osaka S, Hiroshi Y, Goto M, Nagano N, et al. Comparison of characteristics of *Streptococcus dysgalactiae* subsp. *equisimilis* isolates causing repetitive vs single infections. *Ann Lab Med* 2019;39:488–92.
- [8] Fukushima Y, Tsuyuki Y, Goto M, Yoshida H, Takahashi T. Species identification of β -hemolytic streptococci from diseased companion animals and their antimicrobial resistance data in Japan (2017). *Jpn J Infect Dis* 2019;72:94–8.
- [9] Shibayama A, Yoshizaki T, Tamaki M, Goto M, Takahashi T. Pyogenic sternoclavicular arthritis caused by *Streptococcus agalactiae* in an elderly patient with diabetes mellitus. *J Am Geriatr Soc* 2016;64:1376–7.
- [10] Manning SD, Ki M, Marrs CF, Kugeler KJ, Borchardt SM, Baker CJ, et al. The frequency of genes encoding three putative group B streptococcal virulence factors among invasive and colonizing isolates. *BMC Infect Dis* 2006;6:116.
- [11] Spellerberg B, Rozdzinski E, Martin S, Weber-Heynemann J, Schnitzler N, Lütticken R, et al. Lmb, a protein with similarities to the Lral adhesin family, mediates attachment of *Streptococcus agalactiae* to human laminin. *Infect Immun* 1999;67:871–8.
- [12] Bergseng H, Bevanger L, Rygg M, Bergh K. Real-time PCR targeting the sip gene for detection of group B streptococcus colonization in pregnant women at delivery. *J Med Microbiol* 2007;56:223–8.
- [13] Yan SS, Fox ML, Holland SM, Stock F, Gill VJ, Fedorko DP. Resistance to multiple fluoroquinolones in a clinical isolate of *Streptococcus pyogenes*: identification of *gyrA* and *parC* and specification of point mutations associated with resistance. *Antimicrob Agents Chemother* 2000;44:3196–8.

- [14] Takahashi T, Ubukata K, Watanabe H. Invasive infection caused by *Streptococcus dysgalactiae* subsp. *equisimilis*: characteristics of strains and clinical features. *J Infect Chemother* 2011;17:1–10.
- [15] Rodriguez JR, Hsieh F, Huang CT, Tsai TJ, Chen C, Cheng MH. Clinical features, microbiological epidemiology and recommendations for management of cellulitis in extremity lymphedema. *J Surg Oncol* 2020;121:25–36.
- [16] Binnick AN, Klein RB, Baughman RD. Recurrent erysipelas caused by group B streptococcus organisms. *Arch Dermatol* 1980;116:798–9.
- [17] Del Giudice P, van der Mee-Marquet N, David-Rubin F, Le Duff F, Benchia K, Counillon E, et al. Severe relapsing erysipelas associated with chronic *Streptococcus agalactiae* vaginal colonization. *Clin Infect Dis* 2006;43:e67–70.
- [18] Sendi P, Graber P, Johansson L, Norrby-Teglund A, Zimmerli W. *Streptococcus agalactiae* in relapsing cellulitis. *Clin Infect Dis* 2007;44:1141–2.
- [19] Gibson RL, Lee MK, Soderland C, Chi EY, Rubens CE. Group B streptococci invade endothelial cells: type III capsular polysaccharide attenuates invasion. *Infect Immun* 1993;61:478–85.
- [20] Stevens DL, Bisno AL, Chambers HF, Everett ED, Dellinger P, Goldstein EJ, et al. Practice guidelines for the diagnosis and management of skin and soft-tissue infections. *Clin Infect Dis* 2005;41:1373–406.