



# First Case in Korea of Group B Streptococcus With Reduced Penicillin Susceptibility Harboring Amino Acid Substitutions in Penicillin-Binding Protein 2X

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Dear Editor,

*Streptococcus agalactiae* (group B streptococcus, GBS) is the leading cause of neonatal invasive infections, such as sepsis and meningitis [1]. Adult GBS infections can occur as postpartum infections or in immunocompromised patients with underlying diseases, and common clinical syndromes associated with GBS infection are bacteremia, pneumonia, osteomyelitis, and arthritis [2]. Penicillin is the preferred choice for intrapartum antimicrobial prophylaxis and the treatment of GBS infections [3]. Although GBS is mostly susceptible to penicillin, clinical isolates with reduced susceptibility to penicillin or ampicillin have been reported in Japan (*S. agalactiae* strains B1, B6, B7, B8, B10, B12, B40, B60, B68, B502, B503, B513, B514, and B516), North America (GBS strain 860703), and Mozambique [4-6]. We report two GBS isolates with reduced penicillin susceptibility (PRGBS) harboring amino acid substitutions in penicillin-binding protein 2X (PBP2X) isolated from two adult patients [5]. To the best of our knowledge, this is the first report on PRGBS in Korea. This study was approved by the Institutional Review Board (KU1200106) of Konkuk University Medical Center, Seoul, Korea, which waived the need for written informed consent from

the patients.

Case 1: A 76-year-old Korean woman presented with dyspnea and mild fever. She had been suffering from multiple diseases, including type 2 diabetes and Alzheimer's dementia, and had been treated with antimicrobials for septic shock in a long-term care facility. At admission, a coarse breathing sound with rale was auscultated in her both lungs, and a computed tomography (CT) scan of her chest revealed signs of pneumonia. Numerous gram-negative bacilli and gram-positive cocci were grown on her sputum culture. Grayish colonies with beta hemolysis on a blood agar plate were identified as *S. agalactiae* using a Vitek 2 gram-positive (GP) identification system (bioMérieux, Durham, NC, USA) with 93% probability. The *S. agalactiae* isolate tested positive in a Christie-Atkins-Munch-Petersen (CAMP) test.

Case 2: An 87-year-old Korean woman presented with fever, cough, and sputum for several days. She was treated with tazobactam/piperacillin in a long-term care facility for at least five days according to medical records. She suffered from dementia, Parkinson's disease, and intraventricular hemorrhage. A coarse breathing sound with crackle was auscultated in both lungs, and a chest CT scan revealed signs of pneumonia. From sputum

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**Table 1.** Minimum inhibitory concentrations ( $\mu\text{g/mL}$ ) of penicillin for the two Korean isolates of group B streptococcus (GBS) with reduced penicillin susceptibility according to different methods

Isolate	Vitek 2 AST-ST01	E-test	Broth microdilution	Sensititre DKMGN
1	0.25	0.38	0.5	0.25
2	0.25	0.25	0.5	0.25

collected on the 23rd day after admission, predominant gram-positive cocci with beta hemolysis were grown and were identified as *S. agalactiae* by using the Vitek 2 GP identification system with 97% probability.

These two isolates were non-susceptible to penicillin according to commercial minimal inhibitory concentration (MIC) tests, including Vitek 2 AST-ST01 and E-test (bioMérieux). However, they were susceptible to ampicillin ( $\text{MIC} \leq 0.25 \mu\text{g/mL}$ ), cefotaxime, ceftriaxone, linezolid, trimethoprim/sulfamethoxazole, and vancomycin, and resistant to erythromycin, clindamycin, levofloxacin, and tetracycline, according to the CLSI guidelines [7]. We subjected both isolates to additional antibiotic susceptibility tests, using the broth microdilution method according to the CLSI and a Sensititre DKMGN panel (TREK Diagnostic Systems, West Sussex, UK). MICs of penicillin for these two isolates by different methods are presented in Table 1.

Species identification for both isolates was confirmed by 16S rRNA sequencing as previously described [8]. The sequence data revealed 99% identity with *S. agalactiae*. Because amino acid substitutions in PBP2X are considered the first step towards  $\beta$ -lactam non-susceptibility, we analyzed the *pbp2x* sequence [9]. The *pbp2x* gene was amplified with PrimeSTAR HS DNA polymerase Pyrobest (Takara Biotechnology Co., Ltd., Dalian, China), and sequencing and data analysis were carried out using an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA, USA), according to the procedures described previously [5]. Amino acid substitutions in PBP2X (G398A, V405A, and Q557E) were identified in both isolates.

The CLSI defines GBS susceptibility to penicillin as an  $\text{MIC} \leq 0.12 \mu\text{g/mL}$  [7]. Non-susceptibility to penicillin is associated with the acquisition of amino acid substitutions near the active-site motifs in the transpeptidase domain of PBP2X, and two amino acid substitutions in PBP2X, V405A and/or Q557E, are regarded as key substitutions [9, 10]. We first isolated two PRGBS from sputum specimens from Korean patients. Both patients were elderly women with suspected pneumonia and underlying diseases. They had been in long-term care facilities. Most of the PRGBS reported in the literature were from elderly patients; in

Japan, most isolates were from sputa of elderly patients, similar to our cases [5]. Both isolates had V405A and Q557E substitutions in PBP2X, supporting the fact that these isolates are PRGBS. Since PRGBS is a very rare phenotype, species identification and susceptibility should be confirmed by accurate tests, and molecular confirmation of critical amino acid substitutions is recommended [9]. Kimura *et al.* [9] proposed PRGBS classification based on amino acid substitutions in PBPs (PBP2X, PBP2B, PBP2A, PBP1B, and PBP1A); they suggested that amino acid substitution in PBP2X is key and the first step towards  $\beta$ -lactam non-susceptibility. Additional data on molecular subtypes are needed to clarify microbiological and clinical characteristics of each isolate. Further research on PRGBS from Korea will lead to the characterization of  $\beta$ -lactam susceptibility as well as molecular and clinical features in Korean isolates.

### Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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