Published in final edited form as:

Clin Infect Dis. 2020 June 24; 71(1): 201–204. doi:10.1093/cid/ciz1000.

Streptococcus pyogenes pbp2x Mutation Confers Reduced Susceptibility to β-Lactam Antibiotics

Kirsten S. Vannice[1,2], **Jessica Ricaldi**[3], **Srinivas Nanduri**[3], **Ferric C. Fang**[4], **John B. Lynch**[4], **Chloe Bryson-Cahn**[4], **Theodore Wright**[4], **Jeff Duchin**[2], **Meagan Kay**[2], **Sopio Chochua**[3], **Chris A. Van Beneden**[3], **Bernard Beall**[3]

[1]Epidemiology Workforce Branch, Division of Scientific Education and Professional Development, Epidemic Intelligence Service, Center for Surveillance, Epidemiology, and Laboratory Services, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

[2]Communicable Disease Epidemiology and Immunization, Prevention Division, Public Health–Seattle & King County, Seattle, Washington, USA

[3]Respiratory Diseases Branch, Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

[4]Harborview Medical Center, Seattle, Washington, USA

Abstract

Two near-identical clinical *Streptococcus pyogenes* isolates of *emm* subtype *emm43.4* with a *pbp2x* missense mutation (T553K) were detected. Minimum inhibitory concentrations (MICs) for ampicillin and amoxicillin were 8-fold higher, and the MIC for cefotaxime was 3-fold higher than for near-isogenic control isolates, consistent with a first step in developing β-lactam resistance.

Keywords

group A *Streptococcus*; antimicrobial resistance; β-lactam resistance

Streptococcus pyogenes (group A *Streptococcus* [GAS]) causes an estimated 1.8 million severe infections and 517 000 deaths globally every year [1]. The β-lactam antibiotics penicillin and amoxicillin are the antibiotics of choice to treat most GAS infections.

Correspondence: K. Vannice, Centers for Disease Control and Prevention, 1600 Clifton Road NE, MS V 24-5, Atlanta, GA 30329-4027 (nrb8@cdc.gov).

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Potential conflicts of interest. F. C. F. reports grants, personal fees, and nonfinancial support from BioFire and Cepheid, personal fees from the Infectious Diseases Society of America, and grants and nonfinancial support from EliTech, outside the submitted work. All other authors report no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

No confirmed reports of GAS resistance to β-lactam antibiotics have been documented, although treatment failures have been reported [2]. Within *Streptococcus pneumoniae*, "first step" mutations within the penicillin-binding protein PBP2x gene (*pbp2x*) by themselves lead to modestly elevated minimum inhibitory concentrations (MICs) and are required for the development of full β-lactam resistance [3]. *Streptococcus agalactiae* (group B *Streptococcus* [GBS]) and *Streptococcus dysgalactiae* subspecies *equisimilis* have more recently been reported with reduced susceptibility to β-lactam antibiotics conferred by *pbp2x* point mutations [4-6].

In June 2017, Public Health-Seattle & King County began investigating a possible community outbreak of GAS infections at hospital A in collaboration with the US Centers for Disease Control and Prevention (CDC). We report 2 nearly identical GAS isolates, each sharing the same rare mutation leading to elevated β-lactam MICs and each associated with an independent invasive infection.

METHODS

A convenience sample of available GAS blood and wound isolates from unique patients with GAS infections treated at hospital A during May 2017-March 2018 was provided to the CDC. The CDC performed T-protein typing employing type-specific antisera; isolates within the most common T-types were selected for whole genome sequencing-based characterization as previously described [7]. MICs were compared between strains in which point mutations were identified in antibiotic resistance-determining regions, and nearly isogenic strains that lacked these mutations.

For the 2 GAS isolates with the single point mutation associated with higher β-lactam MICs, Public Health-Seattle & King County conducted patient interviews and medical record reviews to determine the course of infection, treatment, and social history during the 3 preceding years.

This project was reviewed in accordance with CDC human research protection procedures and was determined to be a nonresearch, public health response.

RESULTS

During May 2017-March 2018, 282 isolates (44 blood, 6 other sterile fluid, 232 wound) were collected at hospital A from 254 patients. Of 52 isolates sent to CDC, 36 were selected for whole-genome sequencing (Supplementary Table 1).

Five isolates of *emm* subtype 43.4 and multilocus sequence type 3 (*emm43.4*/ST3) from patients A-E revealed an average pairwise single-nucleotide polymorphism (SNP) difference of 6.8 (range, 2–10), indicating close relatedness (Supplementary Figure 1). Four of the 5 patients were identified as experiencing homelessness at the time of isolate collection; the fifth patient worked as a provider of services to people living homeless in the community. Four of the 5 reported using injection drugs.

Of the 5 *emm43.4*/ST3 isolates, 2 differed by only 2 SNPs. These isolates (collected from patient A in November 2017 and patient E in January 2018) showed a single point mutation within *pbp2x*, resulting in the PBP2x T553K substitution (Figure 1). Both isolates also showed a single point mutation within *parC*, resulting in the S79F substitution within the topoisomerase subunit ParC. MIC values were 0.25 μg/ mL for the 2-amino-group containing antibiotics ampicillin and amoxicillin (Table 1), which were 8 times higher than the MIC values for the other 3 nearly isogenic *emm43.4*/ST3 isolates and at the susceptibility breakpoint for ampicillin. MIC values were 0.06 μg/mL for cefotaxime, which was twice as high as the control strain MICs. These were independently verified by utilizing Etest. Penicillin MICs were the same across isolates and below the susceptibility breakpoint (MIC ≤0.015). Intermediate levofloxacin resistance was also detected in the isolate from patient E (MIC = 4 μg/mL) (Supplementary Table 1).

Case 1

Patient A is an adult male with a congenital syndrome associated with poor lower extremity lymph drainage complicated by multiple episodes of septic arthritis of the knee. Patient A presented to hospital A in septic shock on 4 November 2017. GAS was isolated from the patient's blood and knee synovial fluid. He was initially treated with intravenous vancomycin, clindamycin, levofloxacin, and penicillin G; this was narrowed to penicillin G and clindamycin when culture results became available. Patient A was discharged 19 days after admission to complete an outpatient course of levofloxacin. At the time of admission, patient A worked at a facility providing services to people experiencing homelessness in Seattle.

In the 3 years before this hospitalization, patient A had at least 7 invasive bacterial infections caused by GBS, GAS, or methicillin-resistant *Staphylococcus aureus* (MRSA). As a result of these infections, patient A had received at least 9 courses of β-lactam antibiotics (ampicillin-sulbactam, ceftriaxone, penicillin G, and penicillin V potassium), 9 courses of fluoroquinolone antibiotics (levofloxacin and moxifloxacin), and 2 doses of penicillin G benzathine for prophylaxis. Antibiotic courses ranged from a single dose to a 38-day course. The most proximal infection and antibiotic use for patient A was in June 2017, when he received ampicillin-sulbactam and levofloxacin for GBS bacteremia and soft tissue infection.

Case 2

Patient E is an adult male with a history of hepatitis C virus infection and alcoholic cirrhosis. The patient reported a history of injection drug use and was living homeless and unsheltered at the time of GAS infection. He presented to hospital A on 26 January 2018, with 3–4 days of abdominal pain and altered mental status. The patient had large-volume ascites with an ascitic fluid nucleated cell count >10 000 cells/mL and 88% neutrophils. He also had a purulent foot wound. GAS and penicillin-susceptible *S. pneumoniae* were isolated from the blood, and *S. pneumoniae* from ascitic fluid. Patient E was treated with 2 weeks of intravenous ceftriaxone and discharged 14 days after admission.

Patient E had multiple invasive MRSA and *Streptococcus* infections during the 3 years preceding the *pbp2x* mutant isolate collection date, including *Streptococcus dysgalactiae* subspecies *equisimilis*, unspeciated α-hemolytic streptococci, *S. pneumoniae*, and GAS. He had received multiple courses of β-lactam antibiotics (amoxicillin, ceftriaxone, meropenem, cephalexin, and penicillin G) and fluoroquinolone (ciprofloxacin) inpatient and outpatient treatment; courses ranged from a single dose to 30 days. The most proximal infection and antibiotic use for patient E was in November 2017, when he was diagnosed with *S. pneumoniae* bacteremia resulting in treatment with vancomycin, clindamycin, meropenem, cefdinir, and sulfamethoxazole-trimethoprim.

No direct link between patients A and E were established, although patient E reported receiving meals at the same facility serving people experiencing homelessness where patient A worked in the month before the onset of patient E's GAS infection.

DISCUSSION

We describe the first reported GAS clinical isolates with elevated ampicillin, amoxicillin, and cefotaxime MICs conferred by a *pbp2x* mutation. Both isolates were collected from patients with extensive and repeated histories of prior β-lactam use. The GAS PBP2x T553K substitution described here aligns to the same conserved T residue position within GBS (T555) and pneumococci (T550) (Figure 1). In GBS and pneumococci, the corresponding T555S and T550A, respectively, are also associated with reduced susceptibilities to β-lactam antibiotics [3, 5, 8]. This substitution lies immediately following the 3-residue KSG that is 1 of 3 active site motifs conserved in the transpeptidase regions of PBP1a, PBP2b, and PBP2x [9].

Previous research indicates that GAS may be unable to develop β-lactam resistance as a result of barriers to genetic transfer and fitness costs of low-affinity penicillin-binding proteins [10]. We did not see any differences in growth rates between the 2 PBP2x T553K substitution mutants and the 3 closely related control strains (Supplementary Figure 2). GAS isolates with elevated penicillin MICs have been very rarely reported [11, 12], but previous reports have been unconfirmed. Penicillin remains the drug of choice for most GAS infections. Our investigation found no change in penicillin MICs, which is not unexpected given that point mutations reducing the affinity for 1 β-lactam do not necessarily affect the affinity for another [3]. However, here we have shown the first evidence that GAS have the potential to adapt to β-lactam antibiotic selective pressure, as do other streptococcal pathogens, through remodeling of the transpeptidase domain of the key peptidoglycan synthetic enzyme PBP2x.

In exploring the location of the SNPs that were found among this nearly isogenic group of 5 strains, we found 6 SNPs (among the total of only 11 total found within the group) that distinguished the two pbp2x mutants from the 3 "wild-type" strains. Two of these were accounted for by the already-described parC and pbp2x mutations. The four remaining SNPs specific to the 2 pbp2x mutants were missense mutations within four different structural genes. The most compelling of the four was a single mutation within a "glycoside hydrolase family 25" protein (threonine to alanine at position 236, corresponding to protein

QEU36042.1 of reference genome CP044093). The glycoside hydrolases are enzymes that, like PBP2x, are involved in re-modeling peptidoglycan for processes such as cell division and cellular growth. Conceivably, this altered glycoside hydrolase could interact with the peptidoglycan biosynthetic apparatus to functionally complement or alleviate fitness costs of the mutant PBP2x protein.

In view of the close relatedness between the 2 T553K substitution mutants, the *pbp2x* mutation did not arise independently. SNP differences within this cluster of *emm43.4*/ST3 cases indicate that the *pbp2x* mutation emerged within the last 1–2 years. We did not identify a direct link between patient A and patient E, although both had epidemiological links to people experiencing homelessness and overlapped at the same shelter during patient E's exposure period. The detection of this strain in 2 independent invasive infections suggests that it could be widely disseminated. The close relatedness between the 5 isolates that included the 3 wild-type and 2 PBP2x T553K substitution mutants obviated the need for mutagenesis back to wild type for phenotypic comparisons using established protocols [4].

Repeated and prolonged antibiotic use might lead to the emergence of resistant GAS strains. Recent GAS outbreaks have affected people experiencing homelessness, a vulnerable population for which skin and soft tissue infections requiring antibiotic use are common and provide positive selective pressure for mutants to emerge.

We have discovered a novel GAS *pbp2x* mutation conferring elevated MICs to β-lactams, which could serve as a selective advantage and as a first step in development of resistance. These findings reinforce the importance of monitoring β-lactam resistance phenotypes in GAS and adhering to antibiotic stewardship policies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments.

The authors thank Jason Matsumoto and Henry Njuguna for assistance with specimen transfer, and especially thank Ben Metcalf for his work in developing penicillin-binding protein typing for *Streptococcus pyogenes.*

References

1. Carapetis JR, Steer AC, Mulholland EK, Weber M. The global burden of group A streptococcal diseases. Lancet Infect Dis 2005; 5:685–94. [PubMed: 16253886]
2. Sela S, Barzilai A. Why do we fail with penicillin in the treatment of group A Streptococcus infections? Ann Med 1999; 31:303–7. [PubMed: 10574501]
3. Grebe T, Hakenbeck R. Penicillin-binding proteins 2b and 2x of *Streptococcus pneumoniae* are primary resistance determinants for different classes of beta-lactam antibiotics. Antimicrob Agents Chemother 1996; 40:829–34. [PubMed: 8849235]
4. Dahesh S, Hensler ME, Van Sorge NM, et al. Point mutation in the group B streptococcal pbp2x gene conferring decreased susceptibility to beta-lactam antibiotics. Antimicrob Agents Chemother 2008; 52:2915–8. [PubMed: 18541727]
5. Metcalf BJ, Chochua S, Gertz RE, et al. Short-read whole genome sequencing for determination of antimicrobial resistance mechanisms and capsular serotypes of current invasive *Streptococcus agalactiae* recovered in the USA. Clin Microbiol Infect 2017; 23:574.e7–574.e14.

6. Fuursted K, Stegger M, Hoffmann S, et al. Description and characterization of a penicillin-resistant *Streptococcus dysgalactiae* subsp. *equisimilis* clone isolated from blood in three epidemiologically linked patients. J Antimicrob Chemother 2016; 71:3376–80. [PubMed: 27585966]

7. Chochua S, Metcalf BJ, Li Z, et al. Population and whole genome sequence based characterization of invasive group A streptococci recovered in the United States during 2015. mBio 2017; 8. doi:10.1128/mBio.01422-17.

8. Mouz N, Di Guilmi AM, Gordon E, Hakenbeck R, Dideberg O, Vernet T. Mutations in the active site of penicillin-binding protein PBP2x from *Streptococcus pneumoniae.* Role in the specificity for beta-lactam antibiotics. J Biol Chem 1999; 274:19175–80. [PubMed: 10383423]

9. Gordon E, Mouz N, Duée E, Dideberg O. The crystal structure of the penicillin-binding protein 2x from *Streptococcus pneumoniae* and its acyl-enzyme form: implication in drug resistance. J Mol Biol 2000; 299:477–85. [PubMed: 10860753]

10. Horn DL, Zabriskie JB, Austrian R, et al. Why have group A streptococci remained susceptible to penicillin? Report on a symposium. Clin Infect Dis 1998; 26:1341–5. [PubMed: 9636860]

11. Capoor MR, Nair D, Deb M, Batra K, Aggarwal P. Resistance to erythromycin and rising penicillin MIC in *Streptococcus pyogenes* in India. Jpn J Infect Dis 2006; 59:334–6. [PubMed: 17060703]

12. Ogawa T, Terao Y, Sakata H, et al. Epidemiological characterization of *Streptococcus pyogenes* isolated from patients with multiple onsets of pharyngitis. FEMS Microbiol Lett 2011; 318:143–51. [PubMed: 21362024]

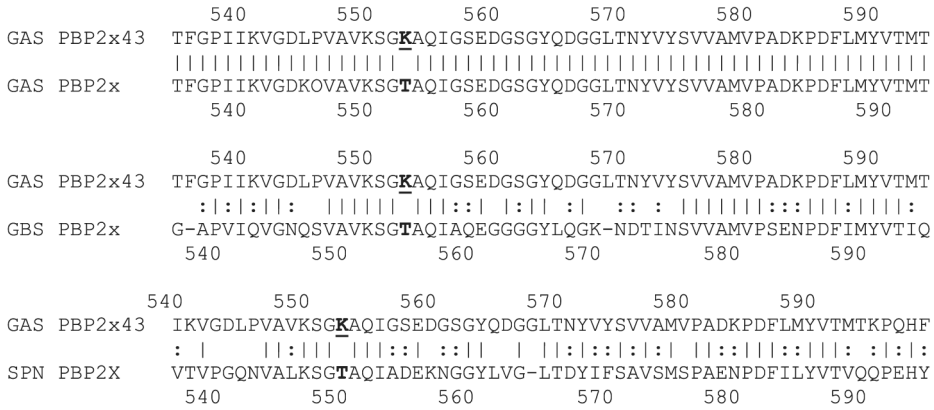

Figure 1.

Alignment of group A *Streptococcus* (GAS) PBP2x segment with wild-type fully susceptible GAS and corresponding segments from susceptible group B *Streptococcus* (GBS) and pneumococci. The 726 residue PBP2x sequence of isolates recovered from patients A and E were identical and differed from PBP2x of nearly isogenic isolates recovered from patients B-D only at amino acid position 553. The conserved T residues corresponding to the GAS PBP2x T553K are shown (T555 in GBS, T550 in *S. pneumoniae* [SPN]). The symbol "|" indicates identical residues, whereas ":" indicates conservative substitutions relative to the GAS PBP2x.

Table 1.

Minimum Inhibitory Concentration Against β-Lactams and Fluoroquinolones of 5 *emm43.4*/Sequence Type 3 Isolates Collected in King County, Washington

	Minimum Inhibitory Concentration, μg/mL							
	β-Lactams (Broth Microdilution Result/Etest Result)						Fluoroquinolones (Broth Microdilution Only)	
Patient ID	**AMP**	**AMX (Etest Only)**	**FOX**	**TAX**	**PEN**	**ZOX**	**CIP**	**LFX**
Patient A	0.25/0.125	0.125	≤2/0.05	0.06/0.023	≤0.015/0.012	≤0.12/ND	4	2
Patient B	≤0.03/0.016	0.016	≤2/0.05	≤0.03/0.008	≤0.015/0.012	≤0.12/ND	1	1
Patient C	≤0.03/0.032	0.032	≤2/0.05	≤0.03/0.006	≤0.015/0.008	≤0.12/ND	2	1
Patient D	≤0.03/0.016	0.016	≤2/0.05	≤0.03/0.008	≤0.015/0.012	≤0.12/ND	1	1
Patient E	0.25/0.125	0.125	≤2/0.05	0.06/0.023	≤0.015/0.012	≤0.12/ND	4	4

Microdilution test results were reproducible between 4 repeated tests (all 5 strains were used for each test). Etests were performed in duplicate.

Source: Clinical and Laboratory Standards Institute (CLSI): https://tinyurl.com/y4a5rxgn.

Abbreviations: AMP, ampicillin (susceptibility breakpoint ≤0.25); AMX, amoxicillin, (no susceptibility breakpoint specified in CLSI guidelines); CIP ciprofloxacin (no susceptibility breakpoint specified in CLSI guidelines); FOX, cefoxitin (no susceptibility breakpoint specified in CLSI guidelines); ID, identifier; LFX, levofloxacin (susceptibility breakpoint ≤2, intermediate resistance 4); ND, not done; PEN, penicillin (susceptibility breakpoint ≤0.12); TAX, cefotaxime (susceptibility breakpoint ≤0.5); ZOX, ceftizoxime (no susceptibility breakpoint specified in CLSI guidelines).