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Original article

CovS modulates the antimicrobial susceptibility of *Streptococcus pyogenes*

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ABSTRACT

In *Streptococcus pyogenes*, the relationship between the major virulent regulatory factors, CovR/S, Mga, and Rgg, and antimicrobial susceptibility has not been elucidated till date. This study aimed to determine whether the inactivation of each regulatory factor affects antimicrobial susceptibility. We used *covS*, *mga*, and *rgg* knockout mutants from 3 different *emm1 Streptococcus pyogenes* strains with respect to the CovS amino acid sequence and investigated their susceptibility pattern to 29 antibiotics. Further, we investigated antimicrobial susceptibility of other 8 *emm1* clinical isolates. The antibiotic susceptibility, particularly the susceptibility to penicillins, of the *covS* mutant strains was greater than that of the wild-type strains and the *mga* and *rgg* mutants. The *covS* complemented strain almost restored the susceptibility. The susceptibility to beta lactam in 1 strain with 2 amino acid sequence differences in CovS was higher than that of the other 2 strains with none or 1 amino acid sequence difference. Eight clinical isolates were classified into 2 groups based on antimicrobial susceptibility which correlate CovS amino acid sequence. The two-component signal transduction sensor protein CovS affects the susceptibility to antibiotics in *Streptococcus pyogenes* via the amino acid difference of CovS sequence.

1. Introduction

The gram-positive bacterium *Streptococcus pyogenes* is one of the most common pathogens causing upper respiratory tract infections. It is also responsible for poststreptococcal diseases such as rheumatic fever and glomerulonephritis, and for severe invasive infections such as streptococcal toxic shock-like syndrome (STSS) and necrotizing fasciitis (Tart et al., 2007).

The production of virulent factors by *Streptococcus pyogenes* is regulated by standalone transcription factors and two-component signal transduction systems (TCSs) (Kreikemeyer et al., 2003). In *Streptococcus pyogenes* SF370, thirteen TCSs have been described, of which CovR/S (also known as CsrR/S) is the most frequently characterized (Graham et al., 2002). CovR/S is reported to be a negative regulatory TCS that directly or indirectly influences the expressions of 10%–15% of S. pyogenes genes, including several virulence factors (Shelburre et al., 2005).

The standalone transcription factor Mga activates the expressions of several virulent genes in an environmentally controlled regulon that includes the genes encoding M protein (*emm*), C5a peptidase (*scpA*), opacity factor (*sof*), and a streptococcal inhibitor of the complement protein (*sic*), when these are present (McIver et al., 1997).

The *rgg* (glycosyltranferase G regulator) gene also encodes a standalone transcriptional regulator that influences the expression of genes associated with virulence, catabolism, and stress responses (Lyon et al., 1998). Inactivation of the rgg gene alters the expression of several known or putative virulence factors including caseinolytic SpeB protease, M protein, C5a peptidase, streptolysin O (SLO), and streptokinase (Chaussee et al., 2006).

Although the relationship between these 3 regulators and the expression of virulent factors has been investigated extensively, their involvement in antimicrobial susceptibility has not yet been elucidated. This study aimed to determine whether the lack of each CovS, Mga, and Rgg affects the susceptibility to antibiotics in *Streptococcus pyogenes*.

2. Materials and methods

2.1. Strains

We studied 11 *emm1 Streptococcus pyogenes* strains. Except for SF370, ten *Streptococcus pyogenes* strains (1529, MDYK, MDMH, D2TY, D1NS, GT01, K2, AP04, AP06, and CR01) were obtained from STSS or pharyngitis patients in Japan (Hasegawa et al., 2010). Of these, we used *covS* (Δ*covS*), *mga* (Δ*mga*), and *rgg* (Δ*rgg*) knockout mutants from *Streptococcus pyogenes* strains SF370, 1529, and GT01 as described elsewhere (Sawai et al., 2007). Mutants with nonpolar inactivated *covS*, *mga*, and *rgg* genes were constructed by double-crossover allelic replacement in the chromosome of *Streptococcus pyogenes* (Sawai et al., 2007). We used the plasmid covS::aad9/pFW12, mga::aad9/pFW12, and rgg::aad9/pFW12 for the *covS*-, *mga*-, *rgg*-, knockout mutant as described elsewhere (Sawai et al., 2007). For the preparation of competent cells, strain 1529 was harvested at early to mid-log phase (OD660, 0.4) and washed twice with 0.5 M sucrose buffer. The suicide vectors, covS::aad9/pFW12, mga::aad9/pFW12, and rgg::aad9/pFW12, were each transformed into strain SF370, 1529, and GT01 by electroporation. The conditions of electroporation were 1.25 kV/mm, 25-μF capacitance, and 200-Ω resistance; and electroporation was performed with a GenePulser II instrument (Bio-Rad, Hercules, CA). After incubation at 37°C for 3 h, competent cells were spread onto brain heart infusion (BHI; Eiken Chemical Co., Tokyo, Japan) agar plates containing 0.3% yeast extract (Difco Laboratories, Detroit, MI) and spectinomycin (final concentration, 100 μg/ml). Selected colonies on the plates were cultured. The cultured bacteria were washed once with saline, resuspended in 10 mM Tris-1 mM EDTA, and boiled for 10 min. Genomic DNA was obtained from the supernatant of the boiled bacteria. The double-crossover replacement was analyzed by PCR of the genomic DNA. Successful double-crossover replacement was further confirmed by DNA sequencing. Furthermore we used *covS* complemented strain 1529Δ*covS*comp and 1529Δ*covS* harbouring empty vector strain 1529Δ*covS*pLz as described

elsewhere (Tatsuno et al., 2013). Bacteria were cultured in BHI containing 0.3% yeast extract for 18–24 h at 37°C before disk diffusion testing or MIC determination.

2.2. Disk diffusion assay

We performed the Kirby-Bauer disk diffusion susceptibility assay by using the clinical laboratory standards institute (CLSI) procedures for the 29 antibiotics (Eiken Kagaku Co., Ltd. Tokyo, Japan); PCG, Penicillin G; AMP, Ampicillin; AMX, Amoxicillin; PIP, Piperacillin; FRPM, Faropenem; CEF, Cefalotin; CFZ, Cefazolin; CEC, Cefaclor; CTM, Cefotiam; CMZ, Cefmetazole; CAZ, Ceftazidime; CFP, Cefoperazone; IPM, Imipenem; MEM, Meropenem; TET, Tetracycline; DOX, Doxycycline; NOR, Norfloxacin; LVX, Levofloxacin; SPX, Sparfloxacin; ERY, Erythromycin; CLR, Clarithromycin; AZM, Azithromycin; JOM, Josamycin; CLI, Clindamycin; VAN, Vancomycin; CHL, Chlorampenicol; LZD, Linezolid; Q/D, Quinupristin/dalfopristin; BAC, bacitracin (Clinical Laboratory Standard Institution, 2006). A Kirby-Bauer disk was placed on a Müeller-Hinton agar plate (Difco Laboratories, Detroit, MI) supplemented with 5% sheep blood, and the zone of inhibition was measured after incubation at 35°C for 18–24 h in a 5% CO_2 atmosphere (Clinical Laboratory Standard Institution, 2006).

2.3. MIC determination

Susceptibility testing was performed using broth micro dilution in Müeller-Hinton broth supplemented with 3% lysed horse blood, in accordance with the CLSI guidelines (Clinical Laboratory Standard Institution, 2006). The antibiotics determined by MIC were penicillin G, erythromycin, clindamycin, and tetracycline (Sigma Chemical Co,.St. Louis, MO.). Quality-control testing was performed with *Streptococcus pneumoniae* ATCC 49619 (Clinical Laboratory Standard Institution, 2006).

2.4. Statistical analysis

The degree of significance between different means was determined by the unpaired t test. A p value of <0.01 was regarded as significant. Each test was repeated at least three times to confirm the reproducibility.

3. Results

3.1. Disk diffusion assay for *covS*, *mga*, and *rgg* mutants

We performed the disk diffusion assay about *covS*, *mga*, and *rgg* mutant strains from 3 *Streptococcus pyogenes* strains whose CovS amino acid sequences were different as described below. Table 1, 2, and 3 presented the results of the disk diffusion susceptibility assay in 3 *Streptococcus pyogenes* wild-type strains (SF370, 1529, and GT01) and their derivative mutant isolates, respectively. The Kirby-Bauer disk diffusion susceptibility testing of the *Streptococcus pyogenes* strains exhibited the same susceptibility to the assessed antibiotics between the wild-type and Δ*mga* strains. The susceptibility between the wild-type and Δ*rgg* isolates was consistent with that in a previous report (Sawai et al., 2007).

However, significant differences were observed in the inhibitory zones by penicillins between the wild-type and Δ*covS* mutants ($p < 0.01$). The difference in the inhibitory zones by cephalosporins and carbapenems between the wild-type and Δ*covS* strains was lower than those by penicillins. The susceptible levels of tetracycline, macrolide, chloramphenicol, linezolid, quinupristin/dalfopristin, and bacitracin in Δ*covS* mutants were also moderately higher than those in wild-type strains. A slight difference in the inhibitory zone by quinolone was observed between the wild-type strains and ΔcovS mutants. No differences were detected in the inhibitory zones by vancomycin.

The susceptibility of antibiotics in wild-type GT01 was higher than those of the other 2 wild-type strains SF370 and 1529. One or two amino acid differences from CovS sequence of SF370 were present in strains 1529 and GT01, respectively (Hasegawa et al., 2010). From these results, we speculated that the variation of susceptible patterns among strains was associated with the differences in the CovS amino acid sequence.

Next, we evaluated the disk diffusion assay for 1529 wild-type and mutant strains. Table 4 showes the results of the disk diffusion susceptibility assay in *Streptococcus pyogenes* wild-type strains 1529, Δ*covS* mutant strain 1529Δ*covS*, *covS* complemented strain 1529Δ*covS*comp and Δ*covS* mutant strain transformed with empty vector 1529Δ*covS*pLZ. The susceptibility pattern in 1529Δ*covS*comp was almost successfully recovered. This effect

depends on *covS* gene itself because the susceptible pattern in empty vector transformed strain was not completely recovered.

Table 1
Comparative in vitro disk diffusion assay of different antimicrobials against SF370 wild-type and *covS*-, *mga*-, and *rgg*-mutant strains of *Streptococcus pyogenes*. Measures are expressed in mm.

	Strains			
Antibiotics	**SF370**	**SF370Δ*covS***	**SF370Δ*mga***	**SF370Δ*rgg***
PCG	28.2±2.1	36.2±2.0 *	28.2±2.2	28.2±2.2
AMP	28.2±2.1	36.4±2.1 *	28.2±2.1	28.2±2.3
AMX	28.2±2.0	36.4±2.0 *	28.2±2.2	28.2±2.2
PIP	28.2±2.2	36.2±2.1 *	28.2±2.1	28.2±2.2
FRPM	24.2±2.2	30.2±2.4	24.2±2.2	24.2±2.2
CEF	26.2±2.2	32.2±2.2	26.2±2.2	26.2±2.4
CFZ	26.2±2.4	32.2±2.2	26.2±2.2	26.2±2.2
CEC	24.2±2.2	30.2±2.2	24.2±2.4	24.2±2.2
CTM	24.2±2.2	30.2±2.4	24.2±2.2	24.2±2.2
CMZ	24.2±2.4	30.2±2.2	24.2±2.2	24.2±2.2
CAZ	26.2±2.2	32.2±2.2	26.2±2.4	26.2±2.2
CFP	26.2±2.4	32.2±2.2	26.2±2.2	26.2±2.2
IPM	32.2±2.2	38.2±2.4	32.2±2.2	32.2±2.4
MEM	32.2±2.2	38.2±2.2	32.2±2.4	32.2±2.2
TET	22.2±2.4	26.2±2.2	22.2±2.2	22.2±2.2
DOX	22.2±2.2	26.2±2.2	22.2±2.4	22.2±2.2
NOR	16.2±2.2	18.2±2.4	16.2±2.2	16.2±2.2
LVX	16.2±2.2	18.2±2.4	16.2±2.4	16.2±2.2
SPX	16.2±2.2	18.2±2.4	16.2±2.2	16.2±2.2
ERY	20.4±2.4	24.4±2.4	20.4±2.2	20.4±2.4
CLR	21.4±2.2	25.4±2.4	21.4±2.4	21.4±2.4
AZM	18.4±2.4	22.4±2.2	18.4±2.4	18.4±2.4
JOM	20.4±2.2	24.4±2.4	20.4±2.2	20.4±2.4
CLI	18.4±2.4	22.4±2.4	18.4±2.4	18.4±2.2
VAN	20.2±2.2	20.2±2.4	20.2±2.2	20.2±2.2
CHL	26.2±2.4	30.2±2.2	26.2±2.2	26.2±2.4
LZD	26.2±2.2	30.2±2.4	26.2±2.2	26.2±2.2
Q/D	26.2±2.2	30.2±2.2	26.2±2.2	26.2±2.2
BAC	28.4±2.4	32.4±2.2	28.4±2.4	28.4±2.2

Data represent the mean value and standard error of the mean.
Asterisks represent $p<0.01$.

Table 2
Comparative in vitro disk diffusion assay of different antimicrobials against 1529 wild-type and *covS-*, *mga-*, and *rgg-*mutant strains of *Streptococcus pyogenes*. Measures are expressed in mm.

	Strains			
Antibiotics	**1529**	**1529Δ*covS***	**1529Δ*mga***	**1529Δ*rgg***
PCG	28.2±2.1	36.2±2.0 *	28.2±2.2	28.2±2.2
AMP	28.2±2.0	36.4±2.1 *	28.2±2.1	28.2±2.3
AMX	28.2±2.1	36.4±2.1 *	28.2±2.2	28.2±2.1
PIP	28.2±2.0	36.2±2.1 *	28.2±2.1	28.2±2.2
FRPM	24.2±2.2	30.2±2.2	24.2±2.4	24.2±2.2
CEF	26.2±2.4	32.2±2.2	26.2±2.2	26.2±2.2
CFZ	26.2±2.2	32.2±2.4	26.2±2.2	26.2±2.2
CEC	24.2±2.2	30.2±2.2	24.2±2.2	24.2±2.4
CTM	24.2±2.2	30.2±2.4	24.2±2.2	24.2±2.2
CMZ	24.2±2.2	30.2±2.2	24.2±2.4	24.2±2.2
CAZ	26.2±2.2	32.2±2.4	26.2±2.2	26.2±2.2
CFP	26.2±2.2	32.2±2.2	26.2±2.4	26.2±2.2
IPM	32.2±2.2	38.2±2.2	32.2±2.2	32.2±2.4
MEM	32.2±2.2	38.2±2.2	32.2±2.4	32.2±2.2
TET	22.2±2.2	26.2±2.4	22.2±2.4	22.2±2.2
DOX	22.2±2.2	26.2±2.2	22.2±2.4	22.2±2.4
NOR	16.2±2.2	18.2±2.4	16.2±2.2	16.2±2.2
LVX	16.2±2.2	18.2±2.2	16.2±2.2	16.2±2.4
SPX	16.2±2.2	18.2±2.2	16.2±2.4	16.2±2.2
ERY	20.4±2.4	24.4±2.2	20.4±2.4	20.4±2.4
CLR	21.4±2.2	25.4±2.4	21.4±2.4	21.4±2.4
AZM	18.4±2.4	22.4±2.4	18.4±2.2	18.4±2.4
JOM	20.4±2.2	24.4±2.4	20.4±2.2	20.4±2.4
CLI	18.4±2.4	22.4±2.2	18.4±2.4	18.4±2.4
VAN	20.2±2.4	20.2±2.2	20.2±2.4	20.2±2.2
CHL	26.2±2.2	30.2±2.4	26.2±2.4	26.2±2.2
LZD	26.2±2.2	30.2±2.2	26.2±2.4	26.2±2.2
Q/D	26.2±2.4	30.2±2.2	26.2±2.2	26.2±2.2
BAC	28.4±2.2	32.4±2.2	28.4±2.2	28.4±2.2

Data represent the mean value and standard error of the mean.
Asterisks represent $p<0.01$.

Table 3
Comparative in vitro disk diffusion assay of different antimicrobials against GT01 wild-type and *covS*-, *mga*-, and *rgg*-mutant strains of *Streptococcus pyogenes*. Measures are expressed in mm.

	Strains			
Antibiotics	**GT01**	**GT01Δ*covS***	**GT01Δ*mga***	**GT01Δ*rgg***
PCG	32.4±2.2	36.4±2.4	32.4±2.2	32.4±2.2
AMP	32.4±2.4	36.2±2.2	36.4±2.4	36.4±2.4
AMX	32.4±2.4	36.2±2.4	32.4±2.4	32.4±2.2
PIP	32.2±2.2	36.2±2.4	32.2±2.2	32.2±2.2
FRPM	28.2±2.2	30.2±2.2	28.2±2.4	28.2±2.2
CEF	28.2±2.2	32.2±2.4	28.2±2.2	28.2±2.2
CFZ	28.2±2.2	32.2±2.2	28.2±2.4	28.2±2.2
CEC	27.2±2.4	30.2±2.2	27.2±2.4	27.2±2.2
CTM	27.2±2.2	30.2±2.2	27.2±2.2	27.2±2.4
CMZ	27.2±2.2	30.2±2.4	27.2±2.2	27.2±2.2
CAZ	28.2±2.2	32.2±2.2	28.2±2. 4	28.2±2.2
CFP	30.2±2.2	32.2±2.4	30.2±2.2	30.2±2.2
IPM	36.2±2.4	40.2±2.2	36.2±2.2	36.2±2.2
MEM	36.2±2.2	40.2±2.2	36.2±2.4	36.2±2.2
TET	24.2±2.2	26.2±2.4	24.2±2.2	24.2±2.2
DOX	24.2±2.2	26.2±2.4	24.2±2.2	24.2±2.2
NOR	16.2±2.2	18.2±2.2	16.2±2.4	16.2±2.2
LVX	16.2±2.4	18.2±2.2	16.2±2.2	16.2±2.2
SPX	16.2±2.2	18.2±2.2	16.2±2.2	16.2±2.4
ERY	22.4±2.4	24.4±2.4	22.4±2.4	22.4±2.4
CLR	23.4±2.4	25.4±2.2	23.4±2.4	23.4±2.2
AZM	20.4±2.4	22.4±2.4	20.4±2.2	20.4±2.4
JOM	22.4±2.4	24.4±2.2	22.4±2.4	22.4±2.4
CLI	19.4±2.4	22.4±2.4	19.4±2.2	19.4±2.4
VAN	20.2±2.2	20.2±2.2	20.2±2.4	20.2±2.4
CHL	28.2±2.2	30.2±2.2	28.2±2.2	28.2±2.2
LZD	28.2±2.2	30.2±2.4	28.2±2.2	28.2±2.2
Q/D	28.2±2.2	30.2±2.2	28.2±2.4	28.2±2.4
BAC	30.2±2.2	32.2±2.2	30.2±2.4	30.4±2.2

Data represent the mean value and standard error of the mean.

Table 4
Comparative in vitro disk diffusion assay of different antimicrobials against 1529 wild-type, 1529 *covS* mutant, 1529 *covS* complemented, and 1529 *covS* mutant with empty vector strains of *Streptococcus pyogenes*. Measures are expressed in mm.

	Strains			
Antibiotics	**1529wild-type**	**1529Δ*covS***	**1529Δ*covS*comp**	**1529Δ*covS*pLZ**
PCG	28.2±2.1	36.2±2.0 *	29.2±2.1	36.2±2.1 *
AMP	28.2±2.0	36.4±2.1 *	29.2±2.0	36.4±2.1 *
AMX	28.2±2.1	36.4±2.0 *	29.2±2.1	36.4±2.0 *
PIP	28.2±2.0	36.2±2.1 *	29.2±2.1	36.2±2.1 *

Data represent the mean value and standard error of the mean.
Asterisks represent $p<0.01$.

3.2. Disk diffusion assay with other 8 clinical strains

Furthermore, we analyzed other 8 *Streptococcus pyogenes* clinical isolates which have one or two amino acid differences in CovS protein. Disk diffusion assay of a total of 8 strains revealed that 2 susceptibility patterns in penicillin G were identified (Table 5). There was no significant difference in the susceptibility pattern between the wild-type CovS amino group and one amino acid different CovS group (Table 5). However, the susceptibility in the two amino acid different CovS group was higher than that in the other 2 groups (Table 5). However, there was no difference of susceptibility among the 2 CovS amino acid mutational group.

3.3. MIC determination

From 29 antibiotics, we chose 4 typical antibiotics (penicilline G, erythromycin, clindamycin, and tetracycline) against *Streptococcus pyogenes* and measured the MICs of 1529, 1529Δ*covS*, 1529Δ*covS*comp, and 1529Δ*covS*pLZ (Table 6). The Δ*covS* mutant strains with increased zones of inhibition of penicillin G, erythromycin, clindamycin, and tetracycline were all found to have decreased MICs of these antibiotics as compared with the wild-type strains. Especially, the MIC of penicillin G in 1529ΔcovS was one-eights times as same as that in 1529 wild-type. The MICs of 4 antibiotics in 1529Δ*covS*comp was almost successfully recovered, too. We found no standard deviations in MIC values compared to the values of inhibition zones in disk diffusion assay. We also found that the MICs of the antibiotics assessed in this study were within the susceptible range.

4. Discussion

This is the first report of the association between the *Streptococcus pyogenes* two component sensor protein CovS and antibiotic susceptibility. We demonstrated that *covS* -inactivation in *Streptococcus pyogenes* was associated with increased antibiotic susceptibility, particularly penicillin susceptibility. In a previous report, the MIC of penicillin G was 0.012 μg/mL for both wild-type and *rgg* mutant strains, and there were no differences in the MIC between these two strains (Sawai et al., 2007). This data was consistent with our results. In our study, beta-lactam antibiotics may be strongly associated with the function of CovS protein.

Various antibiotic stresses have been reported to be recognized by other bacterial TCSs. Certain *Streptococcus mutans* TCS mutant strains have been demonstrated to have increased sensitivity to antibiotics that specifically target cell wall biosynthesis (Biswas et al., 2008) coordinate the genetic response to cell wall-synthesis inhibitory antibiotics such as vancomycin (Mascher et al., 2004). *Staphylococcus aureus* VraSR is induced by bacitracin and other cell-wall-synthesis inhibitory antibiotics. A VraSR deletion strain shows a significant increase in sensitivity to the antibiotics it senses (Kuroda et al., 2003). VraSR controls a large regulon, including target genes with functions linked to cell wall metabolism. Here, the susceptibility to bacitracin, but not vancomycin, in *covS* mutant strains increased. Based on the antibiotic susceptibility pattern, the comparative investigation of TCS among other several bacteria may be also necessary.

We previously divided the emm1 *Streptococcus pyogenes* strains into 3 types based on their NADase activity (Tatsuno et al., 2007). SF370, 1529, and GT01 used here were also classified into 3 different groups based on their NADase activity. Thus, we selected these 3 strains on the basis of the groups to which they belonged. Furthermore, their covS sequences analysis revealed the diversity of the CovS amino acid sequence. Although no differences in susceptibility between wild-type CovS and one amino acid different CovS strains were observed, additional CovS amino acid change could play a role in increasing the antibiotic susceptibility. Although CovS functions were not investigated in 11 strains, our results suggest that the amino acid change in CovS may cause the decreased CovS function that is associated with the antibiotic susceptibility patterns. Although this mechanism has been unclear, two hypotheses are suggested from previous investigations (Graham et al., 2002; Tatsuno et al., 2013). One hypothesis is that CovS may affect the bacterial growth. Previous report showed that a *covS* mutant had lower growth ability than wild-type strain (Tatsuno et al., 2013). Antibiotic stress may result in the wideness of the growth differences between wild-type and covS mutant strains. Another hypothesis is that CovS may affect the function of PBP2a. The *pbp2a* gene encodes transpeptidase which plays a role in crosslinking of cell-wall. Previous report represented that covR mutant increased the expression of *pbp2a* mRNA compared to wild-type strain in microarray study (Graham et al., 2002). CovS regulates the expression of CovR negatively (Tatsuno et al., 2013).

Table 5
Comparative in vitro disk diffusion assay of different antimicrobials against CovS one and two amino acid different *Streptococcus pyogenes*. Measures are expressed in mm.

	Strains							
	CovS one amino acid difference				CovS two amino acid differences			
Antibiotics	**MDYK**	**MDMH**	**D2TY**	**D1NS**	**K2**	**AP01**	**AP06**	**CR01**
PCG	28.2±2.2	28.2±2.4	28.2±2.2	28.2±2.2	32.4±2.4	32.4±2.2	32.4±2.2	32.4±2.2
AMP	28.2±2.2	28.2±2.4	28.2±2.2	28.2±2.4	32.4±2.4	32.4±2.4	32.4±2.2	32.4±2.4
AMX	28.2±2.2	28.2±2.4	28.2±2.4	28.2±2.2	32.4±2.2	32.4±2.4	32.4±2.4	32.4±2.4
PIP	28.2±2.2	28.2±2.4	28.2±2.2	28.2±2.2	32.2±2.2	32.2±2.4	32.2±2.2	32.2±2.2
FRPM	24.2±2.2	24.2±2.2	24.2±2.4	24.2±2.2	28.2±2.4	28.2±2.2	28.2±2.2	28.2±2.4
CEF	26.2±2.4	26.2±2.2	26.2±2.2	26.2±2.2	28.2±2.2	28.2±2.4	28.2±2.2	28.2±2.2
CFZ	26.2±2.4	26.2±2.2	26.2±2.2	26.2±2.2	28.2±2.2	26.2±2.2	26.2±2.4	26.2±2.2
CEC	26.2±2.2	26.2±2.4	26.2±2.2	26.2±2.4	27.2±2.2	26.2±2.4	26.2±2.2	26.2±2.4
CTM	26.2±2.2	26.2±2.2	26.2±2.2	26.2±2.4	27.2±2.4	27.2±2.2	27.2±2.2	27.2±2.2
CMZ	26.2±2.2	26.2±2.2	26.2±2.4	26.2±2.4	27.2±2.2	27.2±2.4	27.2±2.2	27.2±2.4
CAZ	26.2±2.4	26.2±2.2	26.2±2.2	26.2±2.2	28.2±2.2	28.2±2.2	28.2±2.4	28.2±2.2
CFP	26.2±2.4	26.2±2.2	26.2±2.4	26.2±2.2	30.2±2.2	30.2±2.4	30.2±2.2	30.2±2.2
IPM	34.2±2.2	34.2±2.4	34.2±2.2	34.2±2.2	36.2±2.2	36.2±2.2	36.2±2.4	36.2±2.2
MEM	32.2±2.2	32.2±2.2	32.2±2.2	32.2±2.4	34.2±2.2	34.2±2.4	34.2±2.2	34.2±2.2
TET	22.2±2.2	22.2±2.2	22.2±2.4	22.2±2.2	24.2±2.2	24.2±2.2	24.2±2.4	24.2±2.2
DOX	22.2±2.4	22.2±2.2	22.2±2.2	22.2±2.4	24.2±2.2	24.2±2.4	24.2±2.2	24.2±2.2
NOR	16.2±2.2	16.2±2.4	16.2±2.2	16.2±2.2	16.2±2.2	16.2±2.2	16.2±2.4	16.2±2.2
LVX	16.2±2.2	16.2±2.2	16.2±2.4	16.2±2.2	16.2±2.2	16.2±2.4	16.2±2.2	16.2±2.2
SPX	16.2±2.2	16.2±2.2	16.2±2.2	16.2±2.4	16.2±2.2	16.2±2.2	16.2±2.4	16.2±2.2
ERY	20.4±2.4	20.4±2.2	20.4±2.4	20.4±2.2	22.4±2.4	20.4±2.4	22.4±2.2	22.4±2.4
CLR	21.4±2.2	24.4±2.4	21.4±2.2	21.4±2.2	23.4±2.4	23.4±2.2	23.4±2.4	23.4±2.4
AZM	18.4±2.2	18.4±2.2	18.4±2.2	18.4±2.4	20.4±2.2	20.4±2.2	20.4±2.4	20.4±2.2
JOM	20.4±2.4	20.4±2.2	20.4±2.4	22.4±2.4	22.4±2.2	22.4±2.4	22.4±2.4	22.4±2.2
CLI	18.4±2.4	18.4±2.2	18.4±2.2	18.4±2.4	19.4±2.4	19.4±2.2	19.4±2.2	19.4±2.4
VAN	20.2±2.2	20.2±2.4	20.2±2.4	20.2±2.2	20.2±2.2	20.2±2.4	20.2±2.2	20.2±2.2
CHL	24.2±2.4	24.2±2.2	24.2±2.4	24.2±2.2	20.2±2.2	20.2±2.4	20.2±2.2	20.2±2.2
LZD	24.2±2.2	24.2±2.4	24.2±2.2	24.2±2.2	28.2±2.4	28.2±2.2	28.2±2.4	28.2±2.2
Q/D	24.2±2.2	24.2±2.2	24.2±2.4	24.2±2.2	28.2±2.2	28.2±2.4	28.2±2.2	28.2±2.4
BAC	28.4±2.2	28.4±2.4	28.4±2.2	28.4±2.4	30.2±2.2	30.2±2.4	30.2±2.2	30.2±2.4

Data represent the mean value and standard error of the mean.

Table 6
Comparative in vitro MIC assay of 4 different antibiotics against 1529 wild-type, 1529 *covS* mutant, 1529 *covS* complemented, and 1529 *covS* mutant with empty vector strains of *Streptococcus pyogenes*. Measures are expressed in μg/mL.

	Strains			
Antibiotics	**1529wild-type**	**1529Δ*covS***	**1529Δ*covS*comp**	**1529Δ*covS*pLz**
PCG	0.0039	0.00048	0.0039	0.00048
ERY	0.0312	0.0156	0.0312	0.0156
CLI	0.0156	0.0078	0.0156	0.0078
TET	0.0624	0.0312	0.0624	0.0312

Thus the lack of covS may decrease the PBP2a activity via the uptake of *covR* expression and may result in the weakness of cell-wall structure. In this point, further studies regarding this mechanism are warranted.

5. Conclusion

In conclusion, our results demonstrate that one of the two-component sensor proteins, CovS, influences the antimicrobial susceptibility. These results are important steps towards mapping interactions among CovR/S regulatory circuits that control the antimicrobial susceptibility of *Streptococcus pyogenes*.

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Transparency declaration

The authors declare that there is no conflict of interests regarding the publication of this article.

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