A Hyperhemolytic/Hyperpigmented Group B Streptococcus Strain with a CovR Mutation Isolated from an Adolescent Patient with Sore Throat

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Abstract

Group B Streptococci (GBS) are β-hemolytic, gram-positive bacteria that are typically associated with infections in human newborns or immunocompromised adults. However, mutation in the two-component regulator CovR/S relieves repression of hemolysin, potentially increasing virulence of GBS.

We report the isolation of hyperhemolytic/hyperpigmented GBS strain from an adolescent patient who presented to the University of Washington clinic with symptoms of sore throat. While the patient also tested positive for mononucleosis, a GBS strain with increased hemolysis was isolated from the throat swab obtained from the patient. As hyperhemolytic/hyperpigmented GBS strains are typically associated with mutations in the regulator CovR/CovS, we sequenced the covR/S loci in the clinical isolate. An adenine to cytosine mutation resulting in a change in amino acid coding sequence from glutamine at position 120 to proline in CovR (Q120P) was identified. Introduction of the Q120P amino acid substitution in a CovR complementation plasmid abolished complementation of a ∆covR mutant derived from the wild type GBS serotype Ia strain A909; these results confirm that the hyperhemolysis observed in the clinical isolate is due to the Q120P substitution in CovR. Antibiotic was prescribed and the patient’s symptoms resolved without reported complications. This study represents the first report of the isolation of a hyperhemolytic/hyperpigmented GBS strain due to a covR/S mutation from an adolescent patient with persistent sore throat who was also diagnosed with mononucleosis. The isolation of GBS CovR/S mutants indicates their presence in settings of co-infections and includes adolescents.

INTRODUCTION

Group B Streptococci (GBS) are beta-hemolytic, gram-positive bacteria that cause invasive infections in human newborns and elderly adults. Although these bacteria typically exist as commensal organisms in the rectovaginal tract in about 30% of healthy adult women, increasing incidence of invasive GBS infections have been reported in both newborns and elderly adults [1-3]. Common symptoms of GBS infections in non-pregnant adults include bacteremia, skin/soft tissue infections and pneumonia [3].

GBS strains with increased expression of hemolysis and pigmentation have been described [4-8]. Molecular studies...
revealed the existence of a two component regulatory system known as CovR/CovS (CovR/S or CsrR/S) which, down-regulates the expression of many virulence factors including the pluriptotent hemolysin [4-8]. Recently, we showed that hemolysis is due to the ornithine rhamnolipid pigment produced by GBS [9]. GBS isolates with increased expression of the hemolytic pigment due to mutations in CovR/S have been associated with increased virulence [9-12]. Sendi et al., [10] also reported a case of necrotizing fasciitis due to a GBS covR mutant in a 50 year old male. We have also observed that CovR/S mutants accelerated GBS virulence in animal models of adult infections and preterm birth, in a hemolytic pigment dependent manner [11,12]. Although GBS infections in non-pregnant adolescents are relatively uncommon, here, we describe the isolation of a hyperhemolytic/hyperpigmented GBS CovR mutant from an adolescent patient with symptoms of sore throat.

CASE PRESENTATION

A 16-year-old female presented to the University of Washington outpatient clinic complaining of a 3-week history of sore throat. She was a non-smoker and denied any recent antibiotic use. The patient was a febrile, without complaints of fatigue or weight loss, and was noted to have mild pharyngeal erythema and Grade 2+ tonsillar hypertrophy without neck lymphadenopathy on exam. Throat swabs were obtained and sent to the microbiology laboratory for culture to rule out β-hemolytic streptococci, latex agglutination, PathoDX, Remel, which are commonly associated with sore throat infections. Although not routine laboratory practice, the orange pigment production by the organism initiated further investigation. Ultimately, the strain was identified as Streptococcus agalactiae (or Group B Streptococcus, GBS) based on the positive latex agglutination test for the group B antigen. These observations prompted further tests for Group B Streptococcus and we confirmed that orange to red pigmented bacteria were also observed after overnight growth on Granada Media and TSA (see RM003 in Figure 1B & C). Using methods described [13], we then determined that RM003 belongs to GBS capsular serotype II (Figure 1E).

In the GBS strain obtained from the patient with sore throat, we also noted decreased CAMP factor expression apart from increased hemolysis (see RM003 in Figure 1D). Previous studies have indicated that GBS strains with mutations in the two-component system covR/S exhibit increased hemolysis/pigmentation and decreased CAMP factor expression [4,5,8]. We
therefore sequenced the covR/S locus in RM003 as described [9] and compared the sequence to the GBS genome [14]. We identified an adenine to cytosine substitution (A359C) in the CovR sequence of RM003 that resulted in a predicted amino acid change from glutamine at position 120 to proline in CovR (Q120P; CAA-CCA). No mutations were noted in the coding sequence of CovS. Amino acid 120 is located between the receiver and effector domains of CovR; see homology model in [7]. To confirm that the phenotypes demonstrating increased hemolysis and decreased CAMP factor expression can be linked to decreased CovR function, qRT-PCR analysis was performed to assess transcription of key CovR regulated genes such as cylE and cfb as described ([9], also see below). These data revealed that RM003 showed > 50- fold increase in transcription of the gene cylE important for hemolytic pigment biosynthesis [9] and > 15- fold decrease in expression of the CovR activated gene cfb encoding CAMP factor [15] (Figure 2).

To further confirm that the Q120P substitution abolished CovR function in GBS, we also constructed a site directed substitution in a CovR complementation plasmid (pCovR) that was previously shown to complement the ΔcovR strain derived from WT GBS serotype la strain A909 [9]. The results shown in (Figures 3A-C) confirmed that while pCovR complemented GBSA909 ΔcovR for repression of hemolytic activity and pigment and also restored activation of CAMP factor, the pCovRQ120P plasmid failed to do so. We also performed western blots using the GBS CovR antibody as described [11] and confirmed that GBS strains RM003 and A909/pCovRQ120P expressed CovR.
protein (Figure 4). Collectively, these data indicate that a Q120P substitution alleviates CovR function in GBS.

To determine if RM003 is more virulent than WT GBS such as A909, we used the murine sepsis model of infection to compare virulence potential these GBS strains as described as described [11]. The results shown in (Figure 5) indicate that 80% of mice infected with RM003 succumbed to the infection whereas only 20% of mice infected with GBS WT A909 succumbed to the infection.

While it is not possible to conclude that the sore throat infection observed in the patient was solely due to the GBS CovRQ120P strain as mononucleosis was also diagnosed based on a qualitative whole blood Mono Test (OSOM Mono Test, Sekisui diagnostics, San Diego CA.), exacerbation of symptoms observed in the patient may be related to the GBS strain with the covR mutation. As the patient was allergic to penicillin and macrolides, cephalexin was prescribed empirically (bid for 10 days) to treat strep throat and her symptoms resolved without reported complications.

DISCUSSION

Previous studies have shown that hyperhemolytic/hyperpigmented GBS strains with mutations in CovR/S exhibit increased virulence. Both, Sendi et al., and we have observed that GBS CovR/S mutants accelerate mortality in animal models of GBS sepsis and meningitis [10,11]. We further demonstrated that the hyperhemolytic GBS strains are proficient for penetration of human placenta ex vivo, and can be isolated from the amniotic fluid and placental membranes from women in preterm labor [9]. Furthermore, we observed that these strains accelerated fetal injury and preterm birth, in a hemolytic pigment dependent manner, in a pregnant mouse model, [12]. Collectively, these observations emphasize the enhanced pathogenesis of hyperhemolytic/hyperpigmented GBS strains.

GBS clinical isolates with mutations in CovR/S have also been isolated from atypical GBS infections that include a 50 year old adult male with necrotizing fasciitis [10], and elderly patients presenting with either a prosthetic joint infection, conjunctivitis, or urinary tract infection [16]. However, hyperhemolytic/hyperpigmented GBS have not been reported from adolescents presenting with clinical symptoms. Although co-infection with infectious mononucleosis and ß-hemolytic group A streptococci has been reported in children with pharyngitis [17], we are not aware of reports of co-infection with GBS. Taken together, our observations indicate that hyperhemolytic/hyperpigmented GBS clinical isolates with spontaneous mutations in the covR/S locus can be isolated from adolescents presenting with clinical symptoms and co-infections.

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REFERENCES

1. Porta K, Rizzolo D. Preventing group B streptococcal infections in


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