2. Davis GE, Austrian RC, Bell EJ. Observations on *tsutsugamushi* disease (scrub typhus) in Assam and Burma: the recovery of strains of *Rickettsia orientalis*. Am J Hyg. 1947;46:268–86.
3. Khan SA, Dutta P, Khan AM, Topno R, Borah J, Chowdhury P, et al. Re-emergence of scrub typhus in northeast India. Int J Infect Dis. 2012;16:e889–90. http://dx.doi.org/10.1016/j.ijid.2012.05.1030
4. Rathore SK, Dwibedi B, Kar SK, Dixit S, Sabat J, Panda M. Viral aetiology and clinico-epidemiological features of acute encephalitis syndrome in eastern India. Epidemiol Infect. 2014;142:2514–21. http://dx.doi.org/10.1017/S0950268813003397
5. Rahi M, Gupte MD, Bhargava A, Varghese GM, Arora R. DHR-ICMR Guidelines for diagnosis and management of Rickettsial diseases in India. Indian J Med Res. 2015;141:417–22. http://dx.doi.org/10.4103/0971-5916.159279
6. Yang HH, Huang IT, Lin CH, Chen TY, Chen LK. New genotypes of *Orientia tsutsugamushi* isolated from humans in Eastern Taiwan. PLoS One. 2012;7:e46997. http://dx.doi.org/10.1371/journal.pone.0046997
7. Blacksell SD, Tanganuchitcharnchai A, Nawtaisong P, Kantipong P, Laongnualpanich A, Day NPJ, et al. Diagnostic accuracy of the InBios Scrub Typhus Detect enzyme-linked immunoassay for the detection of IgM antibodies in Northern Thailand. Clin Vaccine Immunol. 2016;23:148–54. http://dx.doi.org/10.1128/CVI.00553-15
8. Chowdhury P, Khan SA, Dutta P, Topno R, Mahanta J. Characterization of West Nile virus (WNV) isolates from Assam, India: insights into the circulating WNV in northeastern India. Comp Immunol Microbiol Infect Dis. 2014;37:39–47. http://dx.doi.org/10.1016/j.cimid.2013.10.006
9. Khan SA, Dutta P, Borah J, Chowdhury P, TopnoR, Baishya M, et al. Leptospirosis presenting as acute encephalitis syndrome (AES) in Assam, India. Asian Pac J Trop Dis.2012;2:151–3. http://dx.doi.org/10.1016/S2222-1808(12)60034-6
10. Taylor AJ, Paris DH, Newton PN. A systematic review of mortality from untreated scrub typhus (*Orientia tsutsugamushi*). PLoSNegl Trop Dis. 2015;9:e0003971.http://dx.doi.org/10.1371/journal.pntd.0003971

Address for correspondence: Siraj A. Khan, Regional Medical Research Centre, N.E. Region, Post Box-105, Dist- Dibrugarh, Assam, India; email: sirajkhanicmr@gmail.com

Group B Streptococcal Toxic Shock Syndrome and covR/S Mutations Revisited

Parham Sendi,[1] Muad Abd el Hay,[1] Claudia M. Brandt, Barbara Spellerberg

Author affiliations: University of Bern, Bern, Switzerland (P. Sendi); University of Ulm, Ulm, Germany (M. Abd el Hay, B. Spellerberg); Johann Wolfgang Goethe University, Frankfurt, Germany (C. Brandt)

DOI: http://dx.doi.org/10.3201/eid2301.161063

[1]These authors contributed equally to this article.

Gene mutations in the virulence regulator CovR/S of group A *Streptococcus* play a substantial role in the pathogenesis of streptococcal toxic shock syndrome. We screened 25 group B *Streptococcus* (GBS) isolates obtained from patients with streptococcal toxic shock syndrome and found only 1 GBS clone harboring this kind of mutation.

Streptococcal toxic shock syndrome (STSS) is typically caused by *Streptococcus pyogenes* (group A *Streptococcus* [GAS]) (*1*). Major investigations on host-pathogen interactions have been performed to determine why some persons experience uncomplicated pharyngitis, but STSS develops in others. On a molecular level, mutations in *covS* (a sensor gene of the major virulence regulator CovR/S) have been frequently associated with invasive GAS disease (*2*). In 2009, we reported a case of STSS caused by *S. agalactiae* (group B *Streptococcus* [GBS]) and *covS* mutation (*3*). Here, we reassess those findings in a larger collection of GBS isolates causing STSS.

We tested 26 GBS isolates from 25 patients (22 adults, 3 children) (Table) that were pooled from 3 countries; the United States (22 strains collected 2004–2005), Germany (1 strain, 2006), and Switzerland (2 strains, 2005). For 1 of the 2 case-patients from Switzerland, 2 isolates (same clone) were available for mutation analyses (patient 23 [*4*]). The isolate from our previously published case report (Sweden, 2005 [*3*]) served as a control strain for molecular analyses; the corresponding case-patient was included in the demographic analyses (i.e., 26 patients: 23 adults, 3 children). The median age of the included adult patients was 59 years (interquartile range 45.5–68 years); mortality rate was 35% (8/23). The ages of the 3 children were 0, 30, and 60 days (1 death).

We used standard molecular biology techniques for nucleic acid preparation and analysis. We performed molecular typing by multilocus sequence typing (MLST) as described (*5*) and capsular typing by using latex agglutination and PCR serotype determination (*6*) (Table). To analyze the *cov* gene locus, we amplified the genes *covS* and *covR* by PCR (online Appendix Table, http://wwwnc.cdc.gov/EID/article/22/12/16-1063-Techapp1.pdf). Resulting PCR products underwent DNA sequencing with internal *cov* primers on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Weiterstadt, Germany).

Nucleotide sequence analysis showed that, in 1 of the strains (from patient 18), both genes, the sensor histidine kinase *covS* and the response regulator *covR*, had mutated. In *covR,* at nucleotide position 242, cytosine was replaced by thymine, leading to an amino acid exchange from alanine to valine. In addition, the *covS* gene of this strain showed a 1-bp deletion of adenine at position 895 of the gene, causing a frame shift and leading to a truncated CovS with a stop codon at nucleotide position 926 of the

Table. GBS isolates collected from patients with STSS, showing results of capsular and multilocus sequence typing*

Patient no.	Isolate no.	Patient age	Outcome	Capsular serotype	Sequence type	covR/S mutation
1	BSU286	65 y	Died	II	22	–
2	BSU287	71 y	Survived	Ia	23	–
3	BSU288	46 y	Died	V	1	–
4	BSU289	46 y	Survived	IV	397	–
5	BSU290	43 y	Survived	V	1	–
6	BSU291	35 y	Died	III	19	–
7	BSU292	76 y	Survived	V	1	–
8	BSU293	48 y	Survived	Ia	23	–
9	BSU294	61 y	Died	V	1	–
10	BSU295	45 y	Survived	V	1	–
11	BSU296	63 y	Died	Ib	8	–
12	BSU297	0 d	Survived	Ia	23	–
13	BSU298	70 y	Survived	V	1	–
14	BSU299	60 y	Survived	V	1	–
15	BSU300	73 y	Survived	V	1	–
16	BSU301	66 y	Died	III	17	–
17	BSU302	2 mo	Survived	III	17	–
18	BSU303	94 y	Survived	V	23	Yes
19	BSU304	1 mo	Died	Ia	23	–
20	BSU305	34 y	Survived	V	1	–
21	BSU306	59 y	Survived	IV	2	–
22	BSU307	79 y	Died	Ia	88	–
23 (*4*)	BSU865, VS	38 y	Survived	II	19	–
	BSU866, BC			–	–	–
24	BSU869	32 y	Died	Ib	8	–
25	BSU870	53 y	Survived	Ia	23	–
Control (*3*)	BSU871	50 y	Survived	Ib	8	Yes

*BC, blood culture; GBS, group B *Streptococcus*; STSS, streptococcal toxic shock syndrome; VS, vaginal swab; –, not found.

gene. In the control strain that harbored a 3-bp deletion, our previously published finding was confirmed (*3*). In the remaining strains, *cov* alleles matched the gene sequences of completely sequenced GBS strains in the GenBank database (NEM316, 2603V/R, 909A).

During the past few decades, the overall incidence of invasive GBS infections has increased substantially. This trend is particularly noticeable in the elderly and in persons with co-morbid conditions (*7*). Our results regarding age distribution of patients, mortality rate, and frequencies of different GBS serotypes are in line with results of previous studies. Twelve (52%) of 23 patients were ≥59 years old. The mortality rate for group B STSS (≥30%) was similar to that reported for group A STSS (*1*). In a previous case series, which included 13 patients with group B STSS, the mortality rate was 23% (3/13) (*8*). Three-quarter of our strains (19/26 strains) were attributed to serotype V or Ia/Ib. Large epidemiologic studies have frequently implicated serotype s Ia/Ib, III, and V GBS isolates in the etiology of invasive disease in adults (*9*). Apart from sequence type (ST) 1 and ST23 (15/26 strains), the distribution of MLSTs among the GBS isolates was heterogeneous. The highly virulent GBS lineage ST17 was found in only 2 patients (1 adult and 1 child).

The role of *covR/S* mutations in the switch from colonization to invasion has been demonstrated for GAS in a mouse model (*2*). Consistent with these findings, GAS strains isolated from STSS patients frequently carry mutations in this operon (*10*). Similarly, a 3-bp deletion in the *covR* gene was detected in a GBS strain that caused STSS and necrotizing fasciitis (*3*). However, our investigations on a larger collection of GBS isolates did not confirm a high *cov* mutation rate. Only 1 of 25 GBS clones demonstrated a mutation. From 1 patient, a colonizing and invasive isolate (same clone) was available (*4*); that isolate showed no mutation in *covR/S*.

Our results should be interpreted with caution because the absolute number of included patients is small, and the cases were pooled from various centers. Nonetheless, Ikebe et al. found *covR/S* mutations in 76 (46.3%) of 164 GAS strains causing STSS and in only 1.7% of 59 strains without invasive disease (*10*). In light of these results, the low frequency of mutations found in our collection is surprising. Yet, the association with *covR/S* mutations and GBS TSS in a case report has been shown previously and confirmed here. However, GBS harbors multiple 2-component systems and stand-alone regulators. Our findings indicate that different virulence regulators may be involved in the pathogenesis of fulminant GBS disease.

Acknowledgments

We thank the Active Bacterial Core surveillance collective, including Bernard Beall and the Streptococcus Laboratory, Respiratory Diseases Branch, Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA, for generously providing GBS strains. We also are grateful to Stefanie Mauerer and Beatrice Reinisch for expert technical assistance.

Dr. Sendi is an attending physician and lecturer in infectious diseases at Bern University Hospital and University of Bern, Switzerland. His research interests are group B *Streptococcus* in nonpregnant adults and infections of the locomotor apparatus.

References

1. Stevens DL, Tanner MH, Winship J, Swarts R, Ries KM, Schlievert PM, et al. Severe group A streptococcal infections associated with a toxic shock-like syndrome and scarlet fever toxin A. N Engl J Med. 1989;321:1–7. http://dx.doi.org/10.1056/NEJM198907063210101
2. Walker MJ, Hollands A, Sanderson-Smith ML, Cole JN, Kirk JK, Henningham A, et al. DNase Sda1 provides selection pressure for a switch to invasive group A streptococcal infection. Nat Med. 2007;13:981–5. http://dx.doi.org/10.1038/nm1612
3. Sendi P, Johansson L, Dahesh S, Van-Sorge NM, Darenberg J, Norgren M, et al. Bacterial phenotype variants in group B streptococcal toxic shock syndrome. Emerg Infect Dis. 2009;15:223–32. http://dx.doi.org/10.3201/eid1502.080990
4. Sendi P, Graber P, Johansson L, Norrby-Teglund A, Zimmerli W. *Streptococcus agalactiae* in relapsing cellulitis. Clin Infect Dis. 2007;44:1141–2. http://dx.doi.org/10.1086/513034
5. Jones N, Bohnsack JF, Takahashi S, Oliver KA, Chan MS, Kunst F, et al. Multilocus sequence typing system for group B streptococcus. J Clin Microbiol. 2003;41:2530–6. http://dx.doi.org/10.1128/JCM.41.6.2530-2536.2003
6. Poyart C, Tazi A, Réglier-Poupet H, Billoët A, Tavares N, Raymond J, et al. Multiplex PCR assay for rapid and accurate capsular typing of group B streptococci. J Clin Microbiol. 2007;45:1985–8. http://dx.doi.org/10.1128/JCM.00159-07
7. Ballard MS, Schonheyder HC, Knudsen JD, Lyytikainen O, Dryden M, Kennedy KJ, et al. The changing epidemiology of group B streptococcus bloodstream infection: a multi-national population-based assessment. Infect Dis (Lond). 2016;48:386–91.
8. Al Akhrass F, Abdallah L, Berger S, Hanna R, Reynolds N, Thompson S, et al. *Streptococcus agalactiae* toxic shock-like syndrome: two case reports and review of the literature. Medicine (Baltimore). 2013;92:10–4. http://dx.doi.org/10.1097/MD.0b013e31827dea11
9. Lambertsen LM, Ingels H, Schønheyder HC, Hoffmann S; Danish Streptococcal Surveillance Collaboration Group 2011. Nationwide laboratory-based surveillance of invasive beta-haemolytic streptococci in Denmark from 2005 to 2011. Clin Microbiol Infect. 2014;20:O216–23. http://dx.doi.org/10.1111/1469-0691.12378
10. Ikebe T, Ato M, Matsumura T, Hasegawa H, Sata T, Kobayashi K, et al. Highly frequent mutations in negative regulators of multiple virulence genes in group A streptococcal toxic shock syndrome isolates. PLoS Pathog. 2010;6:e1000832. http://dx.doi.org/10.1371/journal.ppat.1000832

Address for correspondence: Barbara Spellerberg, Institute of Medical Microbiology and Hygiene, University of Ulm, Albert-Einstein-Allee 11, 89081 Ulm, Germany; email: barbara.spellerberg@uniklinik-ulm.de

Whole-Genome Characterization of a Novel Human Influenza A(H1N2) Virus Variant, Brazil

Paola Cristina Resende, Priscila Silva Born, Aline Rocha Matos, Fernando Couto Motta, Braulia Costa Caetano, Maria do Carmo Debur, Irina Nastassja Riediger, David Brown, Marilda M. Siqueira

Author affiliations: Oswaldo Cruz Institute, Rio de Janeiro, Brazil (P.C. Resende, P.S. Born, A.R. Matos, F.C. Motta, B.C. Caetano, D. Brown, M.M. Siqueira); Central Laboratory of the State of Parana, Curitiba, Brazil (M.C. Debur, I.N. Riediger)

DOI: http://dx.doi.org/10.3201/eid2301.161122

We report the characterization of a novel reassortant influenza A(H1N2) virus not previously reported in humans. Recovered from a a pig farm worker in southeast Brazil who had influenza-like illness, this virus is a triple reassortant containing gene segments from subtypes H1N2 (hemagglutinin), H3N2 (neuraminidase), and pandemic H1N1 (remaining genes).

Influenza A(H1N2) viruses have been described in human, avian, and especially swine populations over many years (*1,2*). In contrast to the widespread circulation of seasonal H1N1 and H3N2 viruses, subtype H1N2 has been observed only sporadically in humans (*1,3–7*). Human H1N2 infections were reported during 1988–89 from sporadic cases over the winter in China (*3*). In 2000, another H1N2 subtype strain emerged in the human population and became widespread in Europe, with sporadic cases reported in the Middle East, Asia, Africa, and the Americas during 2001–2003 (*1,4*). In Brazil, this H1N2 subtype strain was detected in humans in the southeast region during the winter of 2002 and in the northern region at the beginning of 2003 (*5*). This 2000–2003 H1N2 subtype strain had a genetic origin similar to the 1988–1989 H1N2 strain from China, both reassortants between human seasonal H1N1 and H3N2 subtype lineages (*3,4*).

In contrast, sporadic cases of zoonotic human infections with swine-origin H1N2 subtype variants (H1N2v) have also been described (*6,7*). In Brazil, the passive monitoring of influenza A viruses in pigs has taken place since 2009 (*8*). Recently, a phylogenetic study revealed that H1N2 subtype viruses have circulated undetected in swine herds in Brazil for more than a decade, and reassortments may have occurred (*9*). These viruses seem to be reassortants originating from an ancestor virus introduced to