Int J Clin Exp Med 2016;9(2):3804-3807
www.ijcem.com /ISSN:1940-5901/IJCEM0017761

Original Article

Sequence type 17 *Streptococcus agalactiae* with unusual 'shell-like' phenotype was detected with Xpert® GBS assay

Vincenzo Savini[1], Alessia Franco[2], Roberta Marrollo[1], Roberta De Filippis[1], Nicola Pietro Consilvio[3,4], Valentina Donati[2], Monica Imperi[5], Marco Pataracchia[5], Alessandro Santarelli[6], Paolo Fazii[1], Antonio Battisti[2], Roberta Creti[5]

[1]Clinical Microbiology and Virology, Spirito Santo Hospital, Pescara, Italy; [2]Direzione Operativa Diagnostica Generale, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Rome, Italy; [3]Department of Pediatrics, San Timoteo Hospital, Termoli, Italy; [4]Department of Internal Medicine and Public Health, San Salvatore Hospital, University of L'Aquila, L'Aquila, Italy; [5]Istituto Superiore di Sanità, Dipartimento di Malattie Infettive, Parassitarie e Immunomediate, Rome, Italy; [6]Obstetrics and Gynecology, Ss. Annunziata Hospital, Chieti, Italy

Received October 13, 2015; Accepted January 27, 2016; Epub February 15, 2016; Published February 29, 2016

Abstract: We report the isolation of a *Streptococcus agalactiae* (group B *Streptococcus*, GBS) strain showing a previously undescribed morphology. The isolate was collected during antenatal screening for GBS and formed colonies with irregular shapes and edges, along with a rough surface. Hence, we labeled it as 'shell-like'. Although this strain was not recognized by eye as being GBS, given its unusual features, the Cepheid Xpert® GBS assay (Sunnyvale, US) allowed us to timely provide identification of it as *S. agalactiae*. The strain possessed the capsular serotype III and belonged to Sequence Type 17, which is the most represented in neonatal invasive infections. Unexpected GBS phenotypes may be responsible for falsely negative antenatal screening results, then for an increased risk for neonate disease and, consequently, newborn mortality.

Keywords: *Streptococcus agalactiae*, GBS, group B *Streptococcus*, Lancefield group B, pregnant, pregnancy, gestation, neonate, newborn, Xpert® GBS, Cepheid

Introduction

Streptococcus agalactiae (group B *Streptococcus*-GBS) may occasionally inhabit the human enteric tract from which, in 30% of cases, it reaches the female genital mucosa [1, 2]. Consequently, pregnant women can be GBS silent carriers, who potentially transmit the organism to the neonate, during delivery [1, 2]. Unless intrapartum antibiotic prophylaxis (IAP) is administered, based on intravenous penicillin or ampicillin, 1-2% of newborns may develop an early GBS infection, with sepsis, pneumonia and meningitis. The CDC (Centers for Disease Control and Prevention) therefore recommended to perform rectovaginal cultures at 35th-37th week of gestation, so GBS-positive women will receive IAP when labor starts or as soon as amniotic membranes rupture occurs [1, 2].

GBS is β-hemolytic, less commonly non-hemolytic (γ-hemolytic), even more rarely α-hemolytic [3]. Colonies it forms are circular, convex, smooth, with regular edges [3]. The organism possesses the Lancefield group B antigen and is, in most of cases, CAMP-positive [4].

Based on the capsular polysaccharide (CPS) expressed, 10 bacterial serotypes (Ia, Ib, II-IX) have been described thus far, that are identified by serological and molecular typing methods for epidemiological purposes [5].

Materials and methods

A pregnant woman underwent antepartum screening for GBS [1] at the Spirito Santo Hospital of Pescara, Italy. A rectovaginal swab was plated onto Trypticase Soy agar (TSA-

Figure 1. Nonhemolytic, rough, irregular, indented colonies ('shell-like') formed by strain GBS 492.

Figure 2. GBS 492 small-sized colonies.

Liofilchem®, Roseto degli Abruzzi, Italy) after an overnight incubation in selective enrichment broth (Todd Hewitt broth, Liofilchem®). CAMP test, latex agglutination assay with Lancefield group B antiserum (Liofilchem® Strepto B latex kit) as well as identification through Vitek2 GP card (bioMérieux, Marcy l'Etoile, France) and real-time polymerase chain reaction (RT-PCR)-based Xpert® GBS assay (Cepheid, Sunnyvale, US) [6] were performed, also, on the grown isolate.

Xpert® GBS assay was carried out based on a modified protocol. The test is designed to be used with rectovaginal swabs, in fact; after collection, these are introduced into an Xpert® GBS cartridge, according to the manufacturer's indications. In this occasion, instead, we picked-up a single, pure colony from an overnight culture and put it into a cartridge, being the latter subsequently inserted into the instrument. *S. agalactiae* ATCC 13813 and *Enterococcus faecalis* ATCC 29212 were used as positive and negative controls, respectively.

Additionally, a 16S rRNA gene 428-bp amplicon was analyzed by using CLC DNA Workbench 5.5 and BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and serotype was investigated with a latex agglutination test (Statens Serum Institut) and a multiplex PCR assay [7]. MLST (Multi Locus Sequence Typing) (http://pubmlst.org/sagalactiae/), finally, was used to define the Sequence Type (ST) [8].

Antibiotic susceptibilities were obtained by using Vitek2 AST-ST01 card (bioMérieux) as well as by performing a disc diffusion test according to EUCAST (European Committee for Antibiotic Susceptibility Testing) 2015 guidelines (1 unit benzylpenicillin and 15 µg erythromycin discs as well as Mueller-Hinton plus defibrinated 5% horse blood and 20 mg/mL β-NAD were provided by Liofilchem®).

Results

Culture grew flat, large, non-hemolytic colonies with indented edges within 24 h incubation at 35±1°C under microaerophilic condition (**Figure 1**).

The isolate was presumptively considered as non-GBS, given its morphology, and labeled as an innocent bystander. Nevertheless, and unexpectedly, both CAMP test and group B latex agglutination gave positive results, suggesting we were maybe in front of an unusual GBS phenotype.

Accordingly, identification as *S. agalactiae* was obtained with Vitek2 as well as Xpert® GBS; also, 16S rRNA gene sequencing provided a BLAST 100% homology with *S. agalactiae* strain ATCC 13813 (accession number NR_115728.1).

We wanted to name this morphology as "shell-like", and the organism was stored into the internal collections at the Microbiology and Virology Laboratory, Spirito Santo Hospital of Pescara, Italy (accession number GBS 492), at the 'Istituto Superiore di Sanità, Dipartimento di Malattie Infettive, Parassitarie e Immunomediate', Rome, Italy (accession number ISS9129), as well as at the 'Istituto Zooprofilattico Sperimentale del Lazio e della Toscana', Rome, Italy (accession number 14080541-3).

GBS 492, finally, was observed to possess the serotype III CPS and to belong to the ST17 [8]. To conclude, both betalactams and macrolides were found to be effective, *in vitro*, on the studied strain.

Discussion

This report highlights that attention should be paid when labeling isolates as non-GBS, during pregnancy. Particularly, we depicted what seems to be a previously undescribed phenotype of *S. agalactiae*; in fact, although rough colonies have been seldom reported for this species [9], they have neither been described further nor been shown in pictures, to our knowledge, which could instead help operators to presumptively recognize them in the laboratory activity; also, irregular edges and indented shapes have not been observed previously for *S. agalactiae*, based on the published literature. Again, it was of further interest that, upon subcultures, GBS 492 alternatively formed large- and small-sized colonies, depending on unknown factors (**Figure 2**); both variants displayed, however, a rough morphology with irregular, indented borders. The strain was clearly γ-hemolytic upon overnight incubation; nevertheless, after a 48 h incubation, an exceedingly weak β-hemolytic halo was formed.

It is currently unknown whether and how frequently this GBS phenotype is misrecognized and underreported in the clinical practice. Therefore, in our opinion, latex agglutination and CAMP test should be performed with all Gram positive isolates surviving the overnight selective broth pre-incubation. Nonetheless, even CAMP activity may be decreased in certain strains [10], so it is crucial to timely confirm identification by molecular assays. Unfortunately, PCR methodologies are not uniformly available in clinical laboratories, thus being potentially unattended. Then, strains with unusual morphologies are to be sent to external centers for genome-based identification and this potentially provides results after the infant's birth.

In the light of this, it would be crucial to combine phenotype-based and molecular identification assays in the routine practice, particularly in pregnancy. In this ambit, Xpert® GBS allows an accurate and timely RT-PCR-based GBS detection and, although designed to be used with rectovaginal swabs, it may be precious even when working with colonies, as shown in this occasion [6].

To conclude, isolation of unexpected GBS phenotypes may lead to falsely negative results of the antenatal screening, then to an increased risk for neonate disease and, consequently, newborn mortality. Shell-like, ST17, GBS 492 belongs, in particular, to the clonal complex CC17, a very homogenous group that is overrepresented among isolates causing invasive neonate infections in Italy as well as worldwide [11, 12].

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Vincenzo Savini, Clinical Microbiology and Virology, Spirito Santo Hospital, Via Fonte Romana 8, CAP 65124, Pescara (Pe), Italy. Tel: 0039 340-7379737; E-mail: vincenzo_savini@libero.it

References

[1] Verani JR, McGee L, Schrag SJ; Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention (CDC). Prevention of perinatal group B streptococcal disease--Revised guidelines from CDC, 2010. MMWR Recomm Rep 2010; 59: 1-36.

[2] Creti R, Berardi A, Baldassarri L, Imperi M, Pataracchia M, Alfarone G, Recchia S; GBS Prevention Working Group, Emilia-Romagna and the Neonatal GBS Italian network. Characteristics of neonatal GBS disease during a multicentre study (2007-2010) and in the year 2012. Ann Ist Super Sanita 2013; 49: 370-375.

[3] Manual of Clinical Microbiology 2003. Versalovic J, Carroll KC, Jorgensen JH, Funke G, Landry ML, Warnock DW. ASM press.

[4] Savini V, Franco A, Gherardi G, Marrollo R, Argentieri AV, Pimentel de Araujo F, Amoruso R, Battisti A, Fazii P, Carretto E. Beta-hemolytic, multi-Lancefield antigen-agglutinating *Enterococcus durans* from a pregnant woman, mimicking *Streptococcus agalactiae*. J Clin Microbiol 2014; 52: 2181-2182.

[5] Edmond KM, Kortsalioudaki C, Scott S, Schrag SJ, Zaidi AK, Cousens S, Heath PT. Group B streptococcal disease in infants aged younger than 3 months: systematic review and meta-analysis. Lancet 2012; 379 547-556.

[6] Savini V, Marrollo R, Coclite E, Fusilli P, D'Incecco C, Fazii P. Successful off-label use of the Cepheid Xpert GBS in a late-onset neonatal meningitis by *Streptococcus agalactiae*. Int J Clin Exp Pathol 2014; 7: 5192-5195. eCollection 2014.

[7] Imperi M, Pataracchia M, Alfarone G, Baldassarri L, Orefici G, Creti R. A multiplex PCR assay for the direct identification of the capsular type (Ia to IX) of *Streptococcus agalactiae*. J Microbiol Methods 2010; 80: 212-214.

[8] Jones N, Bohnsack JF, Takahashi S, Oliver KA, Chan MS, Kunst F, Glaser P, Rusniok C, Crook DW, Harding RM, Bisharat N, Spratt BG. Multilocus sequence typing system for group B *Streptococcus*. J Clin Microbiol 2003; 41: 2530-2536.

[9] USMLE® Step 1 QBook. 6th Edition. Kaplan Medical; 2013.

[10] Lupo A, Ruppen C, Hemphill A, Spellerberg B, Sendi P. Phenotypic and molecular characterization of hyperpigmented group B Streptococci. Int J Med Microbiol 2014; 304: 717-724.

[11] Imperi M, Gherardi G, Berardi A, Baldassarri L, Pataracchia M, Dicuonzo G, Orefici G, Creti R. Invasive neonatal GBS infections from an area-based surveillance study in Italy. Clin Microbiol Infect 2011; 17: 1834-1839.

[12] Singh P, Springman AC, Davies HD, Manning SD. Whole-genome shotgun sequencing of a colonizing multilocus sequence type 17 *Streptococcus agalactiae* strain. J Bacteriol 2012; 194: 6005.