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Case Report

CAMP-negative group B *Streptococcus* went unrecognized with Cepheid GeneXpert but was detected by Liofilchem® Chromatic StrepB

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Abstract: A *Streptococcus agalactiae* (group B *Streptococcus*, GBS) strain from antenatal screening was not detected by polymerase chain reaction (PCR)-based Cepheid GeneXpert and was phenotypically CAMP-negative; nevertheless, it formed blue colonies on Liofilchem® Chromatic StrepB, a chromogenic medium which allowed presumptive identification of such an unusual isolate. GeneXpert is a valid, genome-based support in laboratory diagnostics which helps detecting, particularly, nonhemolytic GBS variants that may escape inspection by eye with culture potentially leading to falsely negative results. The system targets the GBS *cfb* gene, encoding the CAMP protein, responsible for the CAMP reaction, which is historically observed in almost all isolates of this species. Such *cfb*-negative strains, that are believed to be rare, appear thereby as both CAMP-negative and PCR-negative thus affecting accuracy of the antenatal screening. Based on the published literature, the one we present here seems to be the first GBS isolate escaping detection with GeneXpert due to strain-specific properties, whereas this system's failure related to low bacterial counts in the rectovaginal sample has been clearly shown elsewhere. Then we emphasize that molecular tests cannot be used alone in the context of GBS carriage screening but should be combined with phenotype-based methods, including chromogenic media, what can contribute making childbirth a glad and safe event.

Keywords: Group B streptococci, GBS, *Streptococcus agalactiae,* pregnancy, gestation, neonate, newborn, GeneXpert, cepheid, *cfb* gene, Chromatic StrepB, Liofilchem®

Introduction

30% of women harbour *Streptococcus agalactiae* (group B *Streptococcus*-GBS) in gut or vagina, that potentially leads to neonate colonization during delivery [1, 2]. Consequently, and unless intrapartum antibiotic prophylaxis (IAP) is given, 1-2% of newborns develop an early GBS infection (sepsis, pneumonia, meningitis), which can be serious, even fatal [1, 2]. The CDC (Centers for Disease Control and Prevention) therefore recommended rectovaginal cultures at 35th-37th week of gestation to select GBS-positive women to whom IAP is offered when labor starts or at the time of amniotic membranes rupture [1, 2].

GBS is β-hemolytic, less commonly non-hemolytic (γ-hemolytic) [3] and ten serotypes (Ia, Ib, II-IX) have been described thus far [4, 5].

Materials and methods

The rectovaginal swab of a pregnant woman undergoing antenatal screening for GBS was incubated overnight in Todd-Hewitt broth (Liofilchem®, Roseto degli Abruzzi, Italy), then plated onto Trypticase Soy Agar (TSA, Liofilchem®) and Chromatic StrepB (Liofilchem®).

Latex agglutination assay with Lancefield group B antiserum (Liofilchem® Strepto B latex kit), a CAMP test and identification through MALDI-TOFF technology-based Vitek MS (bioMérieux,

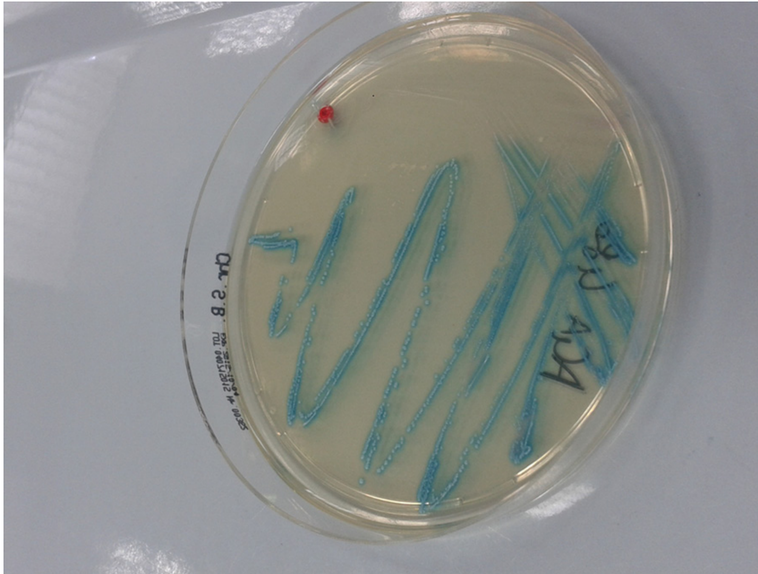

Figure 1. CAMP-negative, GeneXpert-negative GBS480 on Liofilchem® Chromatic StrepB.

Marcy l'Etoile, France) were performed. Also, real-time polymerase chain reaction (PCR)-based Xpert® GBS assay (GeneXpert, Cepheid, Sunnyvale, US) [6] was used to confirm strain's identification.

Such a PCR, particularly, was carried out by picking-up single, fresh colonies from overnight culture and introducing them into the cartridge (provided in the product package) where PCR reactions occur. To enhance test sensitivity, increasing bacterial loads were used (minimum amount: 1 colony, picked up with a sterile loop; maximum amount: a 4 McFarland suspension). *S. agalactiae* ATCC 13813 and *Enterococcus faecalis* ATCC 29212 were used as positive and negative controls, respectively.

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

Results

After 24 h incubation, convex, smooth, β-hemolytic colonies were observed which strongly looked like GBS. Accordingly, the isolate formed blue colonies on Chromatic StrepB (**Figure 1**), which were suggestive for GBS according to Liofilchem® instructions, but it was CAMP-negative, therefore we thought of a falsely positive result of the mentioned medium.

Anyway, a pure colony was picked up and subcultivated, then identified as GBS with Vitek MS. Surprisingly, GeneXpert did not confirm identification, as the system did not provide any amplification of the GBS target region (the *cfb* gene).

16S rRNA gene sequencing nonetheless provided a BLAST 100% nucleotide sequence identity with *S. agalactiae* strain ATCC 13813 (accession number NR_115728.1), thus conclusively confirming identification.

The isolate possessed the serotype V antigen and belonged to the ST1 [8]. It was deposited into the internal collections at the Microbiology and Virology Laboratory, Spirito Santo Hospital of Pescara, Italy (accession number GBS 480) and at the 'Istituto Superiore di Sanità, Dipartimento di Malattie Infettive, Parassitarie e Immunomediate', Rome, Italy (accession number ISS 9337).

Discussion

GBS is the most common cause of early-onset (0-6 days of life) neonatal sepsis in developed countries, where it is acquired intrapartum from women with vaginal and/or rectal colonization; the latter's rates range from approximately 10% to 40%. Intrapartum antibiotics reduce newborn transmission, thereby preventing the early-onset disease. Laboratory detection of GBS in near-term pregnant women is therefore crucial for selective antibiotic prophylaxis prescription at delivery [9].

Guidelines for prevention of early-onset neonatal GBS disease recommend antepartum cultures as screening for rectovaginal colonization

of women at 35-37 weeks of gestation, with intrapartum chemoprophylaxis offered to carriers [1, 9].

To shorten turn-around-time of reporting and increase screening accuracy, highly sensitive molecular assays have been made commercially available, nowadays, that allow rapid detection of antepartum/intrapartum colonization of women who have not been screened prior to delivery or for whom a culture result is not obtained within the time of labor [9]. In this context, the GeneXpert is a valid tool that directly detects the GBS *cfb* gene sequence in rectovaginal samples. Results from this system are obtained within 75 minutes of receipt of the specimen by the laboratory, and GeneXpert is of particular support in the event of carriage of nonhemolytic strains.

When working directly with swabs, reduced GeneXpert sensitivity has been thus far related to initially low GBS counts in the specimen, while invalid and error results may rely on significant amounts of mucus or feces in the tested sample, which inhibit PCR and block the microfluidic channel in the cartridge [10].

In the case we presented, instead, we worked with fresh and pure colonies, thus PCR negative result relied on the lack of the target gene sequence. Accordingly, in fact, the isolate was phenotypically CAMP-negative, as the *cfb* gene notoriously encodes the CAMP factor, as said above.

The CAMP test, first described in 1944, is today used worldwide for presumptive recognition of GBS, and shows an exceedingly high specificity and sensitivity [11, 12]. Particularly, it provides preliminary identification of those isolates that must be addressed to further investigation until identification as GBS is conclusively confirmed or rejected. Although almost all GBS strains are CAMP-positive, CAMP activity may be decreased in certain isolates, what may affect GBS identification in diagnostic laboratories [11, 12]. The test detects a diffusible extracellular protein (the 'CAMP factor') that shows a synergistic hemolysis of sheep erythrocytes in the diffusion zone of staphylococcal β-hemolysin [11, 12].

The CAMP protein was purified and further characterized in 1979, then in 1985. The GBS CAMP factor gene *cfb* has been sequenced by Podbielski, in 1994, and differs from the comparable genes *cfa* of *Streptococcus pyogenes*, *cfu* of *Streptococcus uberis* and *cfg* of *Streptococcus canis*. Moreover, the sequence analysis performed by Podbielski allowed to design *cfb*-specific primers, which have been used for molecular identification of GBS via PCR (i.e. with GeneXpert) [12].

The CAMP factor-encoding *cfb* gene is present in almost all group B streptococci. Nevertheless, isolates which are CAMP factor-negative have been described though believed to be rare [13-15]. Interestingly, CAMP-negative GBS strains with a normal sized *cfb* gene have been observed, too, and the negative CAMP reaction in these isolates could rely on a reduced expression of the CAMP protein, which is however produced [12].

Based on the published literature, the one we presented appears as the first case of GeneXpert failure due to a *cfb*-negative isolate, which was CAMP-negative, accordingly.

As a take-home-message, thereby, we highlight that molecular assays may provide falsely negative results in antenatal screening, due to both sample- and strain-related factors, although epidemiology of genetically mutated GBS strains should be more deeply understood.

We suggest that PCR be not used alone, then; conversely, a rational combination of the culture-based screening at 35-37 weeks of gestation and PCR-based assays could be proposed, to increase screening accuracy; particularly, investigation and research on chromogenic media, like Chromatic StrepB, should be enhanced, to further contribute making childbirth a safe and glad event.

Disclosure of conflict of interest

None.

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