Streptococcus agalactiae serotype Ib as an agent of meningitis in two adult non-pregnant women.

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Two temporally and geographically clustered cases of meningitis caused by

*Streptococcus agalactiae* expressing the infrequent Ib serotype are reported.

Characterization by pulsed-field gel electrophoresis and multilocus sequence typing revealed that the isolates were identical and represented the widely distributed ST10/ST8 lineage associated with serotype Ib.
**Case 1.** A 69-year-old woman, with no known risk factors for group B streptococcal (GBS) infection, was admitted on January 7th 2007 to a tertiary care hospital in Lisbon with headache, nausea and vomiting, disturbances of consciousness and blurred vision with an onset 24 hours prior to admission. On presentation she was apyretic, confused, and she had no signs of meningeal irritation. Hematological investigations revealed a white blood cell (WBC) count of 4,630×10^6/liter (with 85% granulocytes) and a C-reactive protein (CRP) level of 370 mg/liter. A lumbar puncture was performed and blood samples were taken for culture. The CSF exhibited 113/mm^3 WBC (granulocytes), a glucose concentration of 1mg/dl, a protein concentration of 533mg/dl and Gram staining showed gram-positive cocci. A head computed tomography (CAT) scan was unremarkable.

On the basis of initial results empiric therapy, consisting of ceftriaxone (4g daily intravenous) and ampicillin (12g daily intravenous) was started. Clinical evolution was rapid with loss of consciousness and multiple organ failure and the patient died 12 hours after admission.

Both CSF and blood cultures were positive 24h later with *Streptococcus agalactiae* susceptible to penicillin and ceftriaxone.

**Case 2.** Within a few hours of the first case, a second case presented to the same hospital. A 58-year-old woman was admitted with headache, prostration, fever, polyarthralgia and diarrhea with an onset 48 hours prior to admission. On presentation she was comatose, febrile and had positive signs of meningeal irritation. The WBC count
was 17,900 x 10^6/liter (with 88% granulocytes) and CRP level was 292 mg/liter. A lumbar puncture was performed and blood samples were taken for culture. The CSF revealed > 500 WBC/mm^3 (granulocytes), a glucose concentration of < 1mg/dl, protein concentration of 736 mg/dl and the presence of gram-positive cocci. A head CAT scan was unremarkable.

On the basis of initial results empiric therapy, consisting of ceftriaxone (4g daily intravenous) and ampicillin (12g daily intravenous) was started.

Both CSF and blood cultures were positive 24h later with *Streptococcus agalactiae* susceptible to penicillin and ceftriaxone. Based on the microbiological results antimicrobial therapy was changed to penicillin G (24 million units daily intravenously).

Urine culture and vaginorectal swab culture for detection of GBS, as well as another set of blood cultures, performed 24h after admission were all negative. A review of the clinical history revealed a mastectomy due to breast cancer 10 years prior to the current episode as the only possible risk factor for GBS infection. On discharge the patient revealed no motor deficits but was diagnosed neurosensorial deafness and diplopia.

The patients lived on the same street, but interviews of the members of the households were not able to establish a prior social acquaintance. Isolates from both cases were serotyped using specific sera (Denka Seiken, Japan) (5) and a genotypic method (9) as type Ib. Further characterization by pulsed-field gel electrophoresis (PFGE) (11) revealed identical profiles (figure 1). To evaluate if the PFGE profiles were unusual among isolates expressing serotype Ib, 18 isolates from an ongoing nationwide survey of
GBS infections focusing primarily on invasive infections (5, 11) were also characterized by both PFGE and MLST (8) and the results are reported in figure 1.

Although the incidence of GBS invasive infections in non-pregnant adults has increased over the last decades, meningitis remains an uncommon manifestation (3). The incidence of GBS meningitis in adults in the U.S.A. is estimated to be 0.15 cases per 100,000 adults (2) but results in a high case-fatality rate (27-34%) (3). The incidence in Portugal is not known, but a prior study on a large hospital of the Lisbon area did not identify any case (5) and our ongoing nationwide survey started in 2003 identified only three cases, apart from the two reported here, among 916 GBS isolates responsible for infections in adults (136 of those were recovered from normally sterile sites).

The majority of GBS meningitis cases is closely linked with the presence of underlying conditions (2, 4) or to the perinatal period (6) but in some cases no patient risk factors could be associated (1). In some of these cases evidence of an external source of infection was presented (7). In the two cases reported, only the patient of the second case had a known risk factor for GBS invasive infection – a mastectomy following a breast cancer diagnosis. Farley et al. suggest that mastectomy may continue to enhance the risk for GBS invasive infection many years after it was performed, but the presentation of the patients is cellulitis of the arm or chest wall on the side that underwent mastectomy (4). This did not occur in our case and in fact, even if sometimes a distant focus of infection can be established (2), this was not possible in both cases presented. Moreover, in the
second case, vaginorectal colonization could not be established, suggesting an exogenous source.

The temporal and geographical clustering of these two cases prompted further characterization of the isolates. The same serotype (Ib) was identified in both isolates.

Serotype Ib is not among the most frequent serotypes associated with invasive disease in adults in the U.S.A. (3) and our previous study identified a single case of adult bacteremia due to this serotype (n=1/21) (5). Among neonatal infections and colonization of pregnant women in Portugal, serotype Ib was equally unremarkable in prevalence (11). Furthermore, none of the three adult GBS meningitis cases detected by our ongoing survey was associated with this serotype nor were any of the GBS infections in adults identified since January 2007 in the hospital where the two cases were detected (these isolates were serotyped as part of enhanced surveillance triggered by these meningitis cases).

Characterization by PFGE revealed identical profiles (figure 1) indicating that the isolates responsible for these two cases belong to the same bacterial clone. The characterization by PFGE of the isolates in our collection with serotype Ib identified a major cluster and, although no other isolate revealed the same PFGE profile as the isolates responsible for the two cases, these were included in the same cluster as the majority of the Ib isolates. This cluster included isolates associated with colonization and responsible for different clinical presentations in diverse age groups (figure 1). MLST analysis confirmed the distinction of the isolates in two clusters in agreement with the PFGE analysis since two sequence types (ST), ST8 and ST10, are single locus variants and therefore closely related, whereas ST24 differs at 5 out of 7 loci of both of them. Again the isolates
responsible for the two cases presented identical STs – ST10. This was not an unusual
ST among Ib isolates and was found in four additional isolates associated with both
colonization and infection, including invasive disease (figure 1). ST8 and ST10 are also
the most frequent STs found among serotype Ib isolates from various sources and
geographic origins (8, 10). A previous study could not establish an association between a
particular genetic lineage and invasive disease in adults but a significant fraction of
infections caused by clonal complex CC9, where ST8 and ST10 are included, was noted
(10).

Despite not having established a definite common source for the two cases, their temporal
clustering and the proximity of the homes of the two patients strongly suggested that such
common origin could exist. The microbiological findings further support this notion.
Not only did the isolates present an unusual serotype but they also displayed identical
PFGE profiles, albeit belonging to the major genetic lineage associated with serotype Ib.
The virulence of the strain causing these infections is apparent in the fatal outcome of one
of the cases and the neurological sequelae of the surviving case, in spite of appropriate
antimicrobial therapy. GBS is an increasingly significant agent of bacterial meningitis in
adults and the high morbidity and mortality associated with these infections (3), even in
adults with no risk factors, together with the possibility of community outbreaks justifies
their continued monitoring to obtain further insights into this important pathogen.

This work was partly supported by Fundação para a Ciência e Tecnologia (POCI/SAU-
ESP/57646/2004) and by a grant from Fundação Calouste Gulbenkian.
Bibliography


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Figure 1. Characteristics of the GBS isolates responsible for the two cases and of other serotype Ib isolates recovered in Portugal. UPGMA dendrogram using the Dice coefficient of the Smal PFGE profiles of the isolates generated using the Bionumerics software (Sint-Martens-Latem, Belgium). The axis represents the percentage of relatedness between isolates. ST – sequence type as determined by multilocus sequence typing. ND – not determined. Age of the patient is indicated in years (yr) or days (d). The two isolates responsible for the cases reported are highlighted in bold.