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► **To cite this version:**

Arnaud Gagneur, Geneviève Héry-Arnaud, Séverine Croly-Labourdette, Gisèle Gremmo-Feger, Sophie Vallet, et al.. Infected breast milk associated with late-onset and recurrent group B streptococcal infection in neonatal twins: a genetic analysis. *European Journal of Pediatrics*, 2009, 168 (9), pp.1155-1158. 10.1007/s00431-008-0903-y . hal-00557543

HAL Id: hal-00557543

<https://hal.univ-brest.fr/hal-00557543>

Submitted on 21 Jan 2011

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1 **Infected breast milk associated with late-onset and recurrent group B**
2 **streptococcal infection in neonatal twins: a genetic analysis**

3

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24 Acknowledgements

25 We wish to thank Tracey Montagnon for reviewing the manuscript.

26

27 Summary

28

29 Asymptomatic excretion of group B streptococcus (GBS) in breast milk may be
30 an under-recognised cause of neonatal and recurrent infection. We report the
31 case of late-onset and recurrent infection in newborn twins resulting from
32 ingestion of maternal breast milk infected with GBS. Genetic analysis of
33 isolates is equally presented.

34

35 Key-words: group B streptococcus; breast milk; recurrent infection; genetic
36 analysis; late-onset disease

37

38

39 **Introduction**

40

41 Group B streptococcus (GBS) is the most frequent cause of neonatal sepsis
42 and meningitis. Most cases occur in the first week of life and are related to
43 vaginal carriage in the mother (early-onset disease). Conversely, late-onset
44 disease (between 1 week and 3 months of age) is less common and is hand-
45 transmitted by nursery personnel or via other nosocomial or community
46 pathways [6]. Late- onset and recurrent disease have also been reported with
47 the ingestion of infected mother's milk, with some cases confirmed using
48 molecular techniques [2-4, 7, 9, 13]. We report in this article the case of late-
49 onset and recurrent infection in newborn twins resulting from ingestion of
50 maternal breast milk infected with GBS. In this case genetic analysis
51 demonstrated that all GBS isolates (maternal breast milk and vaginal isolates;
52 twin CSF and blood isolates) were identical, but additional genetic analysis
53 also revealed that the GBS isolates were of a particularly virulent clone
54 belonging to serotype III, as are 90% of the strains responsible for late- onset
55 disease [3].

56

57 **Case report**

58

59 Premature twins were delivered by spontaneous vaginal delivery at 31 weeks
60 gestation, 48 hours following membrane rupture. The 26 year- old mother,
61 gravida 1, para 2, received intrapartum antibiotic prophylaxis (single dose of
62 penicillin G) due to positive vaginal GBS culture at 30 weeks gestation. Twin
63 1, female, weighed 1600g with an Apgar score of 10/10 at 1 and 5 minutes.
64 No respiratory failure was noted and early enteral feeding was started with raw
65 breast milk at day 1. Total enteral alimentation with breast milk was obtained
66 at day 6. Twin 2, male, weighed 1720g with an Apgar score of 4/9 at 1 and 5

67 minutes. He was intubated shortly after birth due to respiratory failure and
68 received one dose of surfactant. By the 3rd hour of life, he was extubated and
69 nasal CPAP was initiated. Enteral alimentation with raw breast milk was
70 introduced at day 4. Antibiotic therapy with cefotaxime and amoxicillin was
71 prescribed for both infants due to incomplete prepartum antibiotic prophylaxis
72 and stopped at day 2 due to negative C- reactive protein as well as negative
73 gastric and blood cultures. Cerebral ultrasound examination was normal for
74 both infants at day 4.

75 On day 13, Twin 2 developed cardio-respiratory instability and blood culture
76 tested positive for group B streptococcus. Meningitis was suspected due to
77 elevated CSF protein concentration. Until day 16 Twin 1 was asymptomatic
78 with negative C- reactive protein control. On day 16, she developed
79 respiratory distress and subsequent blood and cerebrospinal fluid cultures
80 tested positive for GBS. Antibiotic treatment with amoxicillin at 200mg/day
81 was prescribed for 14 days, in association with an aminoglycoside during the
82 first 48 hours of treatment. Control blood cultures were negative after day 1, 3
83 and 5 of treatment. Cerebral ultrasound examination controls were normal for
84 both infants. At day 41 of life Twin 1 developed septic syndrome with parotitis
85 and was transferred to the NICU. Blood culture was positive for GBS. Cardiac
86 ultrasound examination was normal. Antibiotic therapy with cefotaxime
87 (200mg/kg/day) and tobramycin (5mg/kg/day) was initiated. At day 7 of
88 infection tomodensitometry examination identified cerebral microabcess and
89 modification of the antibiotherapy ensued, with the administration of
90 ciprofloxacin (30 mg/kg/day) associated with cefotaxime (250 mg/kg/day) for 3
91 weeks. Then oral amoxicillin was initiated for 3 additional weeks. Mastitis was
92 diagnosed in the infants' mother 24 hours following discovery of GBS infection
93 in Twin 1 (day 42). Milk culture tested positive for GBS and the maternal
94 infection was treated with amoxicillin for 10 days. Breastfeeding was

95 suspended and a 10- day preventive oral amoxicillin treatment given to the
96 non- infected twin (confirmed via negative blood and CSF cultures as well as
97 negative CRP controls). Following this infection, the infants remained on
98 pasteurized breast milk. Follow- up at one year showed no cerebral anomalies
99 upon ultrasound examination in association with normal neurological
100 examinations at 1 year of life.

101

102 Analysis revealed all strains as belonging to serotype III. Epidemiological
103 relationships between maternal and neonatal GBS isolates were investigated
104 by pulsed-field gel electrophoresis (PFGE) of DNA restricted with *Sma*I [11].
105 Analysis was conducted on maternal vaginal and raw breast milk isolates (2
106 isolates), a single Twin 2 blood culture isolate (1 isolate), and on Twin 1 CSF
107 and first- and -second blood culture isolates (3 isolates). All six isolates
108 displayed identical PFGE patterns, revealing their genetic relationship (figure
109 1A).

110 Characterization of isolate virulence was conducted by multiplex PCR
111 according to primers and method previously described [11, 12]. First,
112 amplification of the tRNA gene clusters at the 3' end of rRNA operons
113 produced a unique fragment of 1.2 Kb (figure 1B); second, *hyB* amplification
114 produced a 0.3 Kb fragment, showing no *IS1548* insertion within the gene
115 (figure 1B). This pattern was correlated to the invasive phylogenetic division I
116 defined by Musser *et al.* in multilocus enzyme electrophoresis (MLEE)
117 analysis [8, 10].

118

119 **Discussion**

120

121 Cases reporting neonatal late and recurrent group B streptococcal disease
122 associated with raw maternal milk are rare, and few are the studies in which

123 genetic evidence is proposed for this scenario [2, 7, 13]. In our case, not only
124 was total DNA macrorestriction analysis conducted, showing indistinguishable
125 patterns for the six isolates, but additional genetic analysis also revealed that
126 the GBS isolates belonged to a particularly virulent clone shown to produce
127 more extracellular neuraminidase [8]. These isolates, as 90% of the strains
128 responsible for late onset disease [3], belonged to serotype III.

129

130 If the physiopathology of early onset GBS disease is well-documented, little is
131 known about late or recurrent GBS infections. This case offers a novel
132 hypothesis explaining how GBS can cause neonatal infection from 7 days to 3
133 months following delivery.

134 In most of the rare cases described [7, 9], there were no signs of maternal
135 mastitis, indicating a silent maternal duct colonization. Moreover, National
136 Committee of Hygiene guidelines do not systematically screen for GBS in the
137 raw maternal milk supply [1]. These may be two reasons for the
138 underestimation of maternal milk as a source of GBS infection. In the present
139 case, genetic analysis affords evidence for maternal milk as the source of
140 neonatal GBS infection. A circular process was hypothesized by Kotiw *et al*
141 [7]. GBS initially colonizes the neonate's oropharynx mucosa from perinatal or
142 other sources, infecting maternal ducts during breastfeeding. The organism
143 multiplies in the milk ducts. As the microbial concentration increases in the
144 milk, the infant is re-infected during breastfeeding. Mastitis may or may not be
145 present [7]. However Olver *et al* described cases of GBS infection in preterm
146 infants fed with maternal milk via nasogastric tube alone [9]. Prematurity is a
147 recognized predisposing factor to GBS infection although breast milk
148 transmission was also described in term infants [4].

149 In our unit, expressed mother's milk is systematically pasteurized and frozen
150 for conservation in our lactarium, and each specimen of milk is screened for

151 bacteria before administration to preterm infants. However, preterm infants
152 might also receive raw, freshly-expressed breast milk from their mothers
153 present in the unit. When a preterm infant falls clinically ill while the mother is
154 breastfeeding, the mother's milk should be cultured to rule out or to document
155 possible breast milk transmission. Mother's milk feedings should be
156 suspended while providing banked milk pending culture result. If breast milk is
157 positive for GBS, adequate antibiotherapy should also be prescribed for the
158 mother. Byrne et al reported that it is possible to give the mother the
159 opportunity to continue breastfeeding as desired ; she can be encouraged to
160 maintain her milk supply by pumping and discarding milk until appropriate
161 treatment is completed and negative breast milk cultures are obtained [4].

162

163 Asymptomatic excretion of GBS in breast milk may be an under-recognised
164 cause of neonatal and recurrent infection. Recommendations should be
165 established to prevent these infections, notably in the case of multiple births:
166 1/ treatment of both twins; 2 / recognition of the possibility of GBS breast milk
167 infection in late onset or recurrent infection; 3/ suspension of breastfeeding
168 upon suspicion of GBS breast milk infection in both of the children; 4/ search
169 for GBS colonization in both mother and children.

170 However, it should also be noted that use of human milk in the intensive care
171 nursery decreases the incidence of nosocomial sepsis [5] and breastfeeding
172 should still be considered as the most appropriate nutrition for babies and
173 preterm infants [1].

174

175 Conflict of interest statement

176 All authors, no conflict of interest.

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221 Figure 1

222 A. Pulsed-field gel electrophoresis (PFGE) of DNA restricted with *Sma*I of
223 maternal and neonatal GBS isolates. All six isolates displayed identical PFGE
224 patterns, showing their genetic relationship. Molecular weight (MW) ; maternal
225 vaginal isolate (lane 1) ; Twin 1 CSF (lane 2) ; maternal raw breast milk isolate
226 (lane 3) ; first (lane 4) and second (lane 5) Twin 1 blood culture isolates, and
227 Twin 2 blood culture isolate (lane 6).

228 B. Ethidium bromide stain of 2% agarose gel showing multiplex PCR products
229 for the GBS clinical isolates. Amplification of the tRNA gene clusters at the 3'
230 end of rRNA operons produced a unique fragment of 1.2 Kb ; amplification
231 *hy*B gene produced a 0.3 Kb fragment, showing no IS 1548 insertion within the
232 gene. This pattern was correlated to the invasive phylogenetic division I
233 defined by Musser *et al.* in multilocus enzyme electrophoresis (MLEE)
234 analysis [8, 12]. MW, molecular weight ; lane 1, maternal vaginal isolate ; lane
235 2, twin 1 CSF isolate ; lane 3, maternal raw breast milk isolate ; lanes 4 and 5,
236 first and second twin 1 blood culture isolates.

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