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Arnaud Gagneur, Geneviève Héry-Arnaud, Séverine Croly-Labourdette, Gisèle Gremmo-Feger, Sophie Vallet, Jacques Sizun, Roland Quentin, Didier Tandé

► **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

4 Arnaud Gagneur<sup>1,2</sup>, Geneviève Héry-Arnaud <sup>3</sup>, Séverine Croly-Labourdette<sup>1</sup>,  
5 Gisèle Gremmo-Feger<sup>1</sup>, Sophie Vallet<sup>3</sup>, Jacques Sizun<sup>1</sup>, Roland Quentin<sup>4</sup>,  
6 Didier Tandé <sup>3</sup>

7

8 <sup>1</sup>Département de Pédiatrie, Hôpital Morvan, Centre Hospitalo-Universitaire,  
9 Brest, France

10 <sup>2</sup> Département de Pédiatrie, CHUS Sherbrooke, Québec, Canada

11 <sup>3</sup>Département de Microbiologie, EA 3882 Laboratoire Universitaire de  
12 Biodiversité et Ecologie Microbienne, Hôpital Morvan, Centre Hospitalo-  
13 Universitaire, Brest, France ;

14 <sup>4</sup>Service de Bactériologie, Hôpital Trousseau, Centre Hospitalier Régional et  
15 Universitaire, Tours, France ;

16

17

18 Correspondance:

19 Dr Arnaud Gagneur

20 Département de Pédiatrie, Unité de Néonatalogie, CHUS Fleurimont 3001,  
21 12ème avenue nord, Sherbrooke (QC), J1H 5N4, Canada.

22 E-mail: [arnaud.gagneur@usherbrooke.ca](mailto:arnaud.gagneur@usherbrooke.ca)

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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 3<sup>rd</sup> 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 tomodesitometry 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.

177

178



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220

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 *hyf*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.

237

238

239

