**Neonatal Invasive Haemophilus influenzae Disease and Genotypic Characterization of the Associated Strains in Italy**

TO THE EDITOR—We read with interest the article by Collins and colleagues demonstrating that nontypeable *Haemophilus influenzae* (NTHi) is responsible for most invasive disease occurring in the perinatal period and that early-onset neonatal NTHi disease (strain isolation from blood or cerebrospinal fluid within 48 hours of birth) is strongly associated with premature birth [1]. Although it is well known that following the routine use of *H. influenzae* type b conjugate vaccines, most invasive *H. influenzae* disease is caused by NTHi. The value of the Collins’s study was to draw attention to neonatal invasive disease that, to date, has probably been underestimated [2–4]. According to the authors’ conclusions, a better understanding of the responsible NTHi strains through molecular characterization is needed [1].

In Italy, 13 invasive *H. influenzae* disease cases were detected in infants aged ≤31 days through the National Surveillance of Invasive Bacterial Disease (1 January 2009–31 March 2015) (Figure 1, inset). Of 13 infants (all presenting with sepsisemia), 9 (69.2%) were born prematurely (<37 weeks of gestation) and 5 were male. Early-onset invasive infection occurred in 11 neonates (11/13, 84.6%); all but 2 were born prematurely (Figure 1, inset). At discharge, all neonates were alive, but 2 had long-term complications (1 had moderate neurological impairment and 1 had hypoplasia). Looking at maternal factors in early-onset infection, 3 mothers had fever, 3 had chorioamnionitis, and 1 experienced premature rupture of membranes. The 2 neonates who developed late-onset invasive disease (>48 hours after birth) were born at 37 weeks and >38 weeks.

Of 13 *H. influenzae* strains, 10 were sent to the National Reference Laboratory (Istituto Superiore di Sanità, Rome, Italy) where they were characterized. Serotyping,
multilocus sequence typing (MLST), and phylogenetic analysis were performed as previously described [5–8]. All strains were identified as NTHI except for 1 that was found to belong to the “cryptic genspecies of H. influenzae biotype IV,” also referred to as “H. [Haemophilus] quentinii” as previously reported (M. Giufrè and M. Cerquetti, manuscript in preparation). By MLST, each of the 9 NTHi strains belonged to a distinct sequence type (ST; ST11, ST34, ST46, ST142, ST160, ST201, ST425, ST695, and ST834), indicating a high degree of genetic diversity. All but 2 STs (ST11 and ST834) were previously found among invasive NTHi strains in Italy [8]. The “H. quentinii” strain was found to be not typeable by MLST, since no sequence was obtained for the fucK gene. To assess genetic relatedness between the STs herein described and those previously identified among invasive NTHi strains [8], we constructed a phylogenetic tree (Figure 1).

No segregation of the STs from neonatal strains into separate branches was observed. Notably, ST11 clustered together with ST103, ST139, and ST145 (a group of genetically linked STs associated with invasive NTHi disease in Italy [8]) in a separate branch. Actually, ST11 was found as a single-locus variant of ST145 and ST103.

This study documents the occurrence of several cases of neonatal invasive NTHi disease in Italy, too. Previous investigations indicated the “cryptic genspecies biotype IV” as being more capable of causing genital/neonatal infections [9, 10]. Although we recovered a strain belonging to this genspecies, most were NTHi. Our data suggest that NTHi strains responsible for neonatal disease do not constitute a distinct group of related genotypes within the invasive NTHi population. Further studies are required to clarify whether and what makes an NTHi strain capable of causing neonatal invasive infection.

**Notes**

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