Neonatal Group B Streptococcus Infections

Prevention Strategies, Clinical and Microbiologic Characteristics in 7 Years of Surveillance

Roberta Creti, PhD, Monica Imperi, PhD,* Alberto Berardi, MD,† Marco Pataracchia, BSc,* Simona Recchia, PhD,* Giovanna Alfarone, BSc,* and Lucilla Baldassarri, PhD*, on behalf of the Italian Neonatal GBS Infections Working Group*

Background: The characteristics of group B streptococcus (GBS) neonatal disease in a period of 7 years are reported.

Methods: The estimation of the neonatal GBS disease risk and prevention strategies adopted at delivery in absence of national guidelines was evaluated by the analysis of 3501 questionnaires. Notification of 194 neonatal GBS infections was recorded. In addition, 115 strains from neonatal early-onset disease (EOD) and late-onset disease, respectively, plus 320 strains from pregnant women were analyzed by molecular typing methods and for antibiotic resistance.

Results: Preterm deliveries, precipitous labor and GBS negatively screened mothers were the prominent causes for an inadequate or lack of intrapartum antibiotic prophylaxis and EOD occurrence. The superimposable serotype distribution of GBS strains from EOD and from antenatal screening confirmed the vertical transmission from mother to neonate as the cause of disease. On the contrary, late-onset disease was almost exclusively caused by the internationally diffused clonal complex 17. Erythromycin resistance was detected in 17% of strains. Resistance to clindamycin was 15.3 %.

Conclusions: The administration of intrapartum antibiotic prophylaxis to negatively GBS screened women in presence of risk factors was a deviation from the recommendations issued by the Centers for Disease Control and Prevention, and it should deserve further consideration. Routine surveillance and molecular typing of circulating clones are essential for the effective management of the neonatal GBS disease.

Key Words: group B streptococcus, neonatal infection, group B streptococcus antenatal screening, molecular epidemiology, prevention

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Since its emergence in the 1970s, group B streptococcus (GBS) is still the most common pathogen responsible for early-onset sepsis in term and preterm infants and bacterial meningitis in neonates younger than 3 months old in developed countries.[1] Commonly, two patterns of neonatal clinical syndromes caused by GBS have been identified: early-onset disease (EOD) presenting at age 0–6 days and late-onset disease (LOD) affecting infants 7–89 days of age.[2]

The carriage status of pregnant women (PW), which can be asymptomatically colonized with GBS in the vagina and/or rectum (up to 30%), represents the primary risk factor for EOD. Conversely, the predominant routes of LOD transmission are still poorly understood, although maternal GBS carriage and preterm birth can be risk factors.[3,4] Some nosocomial LOD outbreaks[3,5] have been reported, and breast milk has been controversially suggested as possible source for LOD.[6–8]

Prevention is based on intrapartum antibiotic prophylaxis (IAP) to women at risk for vertical GBS transmission. Eligible women can be identified by either antenatal screening culture for GBS colonization (screening-based approach) or assessment of obstetric risk factors during labor (risk-based approach).[9] One of the most compelling evidences of the effectiveness of IAP was the significant reduction in the incidence rates of EOD.[10–15] Unfortunately, antenatal screening and IAP have not changed the incidence of LOD, and no specific strategies are at present available for its prevention.[10]

GBS is traditionally differentiated by ten type-specific capsular polysaccharides (serotypes Ia, Ib, II to IX) and surface protein-family antigens.[16] Serotype III (48.9%) is the most frequent in all regions with available data in Western world followed by serotypes Ia (22.9%), V (9.1%), Ib (7.0%) and II (6.2%).[17] Epidemiologic data on GBS serotype distribution among carriage women and neonatal infections (NIs) in developing or low-/middle- income countries are limited but serotype distribution is similar.[9,18]

The GBS population genetic structure is composed by well-defined clonal complexes (CCs) that are represented both in GBS carriage and disease. Indeed, the CC-17 lineage is a very homogeneous group that is overrepresented among GBS isolates responsible for invasive late-onset infections in neonates.[19] A multicenter study, conducted in Italy in the years 2007–2010, dealt with the collection of information on invasive GBS NIs and the prevention programs adopted by the participating birth centers, in absence of national guidelines.[20] The multicenter surveillance was not maintained and the notification, on voluntary basis, of invasive neonatal GBS infections was launched again in the years 2012–2014. The characteristics of the neonatal GBS infections during the multicenter study and the years 2012–2014 have been compared to evaluate the prominent causes involved in its occurrence and its evolution in 7 years of surveillance and prevention policy.

MATERIALS AND METHODS

Years 2007–2010 Study Outline

An established regional network,[21] plus 9 additional hospitals evenly geographically distributed for a total of about 67,000 births/yr (approximately one-eighth of total annual births in Italy

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From the *Dipartimento di Malattie Infettive, Parassitarie ed Immunomediate, Istituto Superiore di Sanità, Rome, Italy; and †Unità Operativa di Neonatologia, Dipartimento Materno-Infantile, Azienda Ospedaliero-Universitaria, Policlinico di Modena, Modena, Italy.

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Address for correspondence: Roberta Creti, PhD, Reparto di Malattie Batteriche Respiratorie e Sistemiche, Dipartimento di Malattie Infettive, Parassitarie ed Immunomediate, Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161, Rome, Italy. E-mail: roberta.creti@iss.it.

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in that period), participated to the study. Information was collected on prevention strategies adopted at the time of delivery on consecutive births during the years 2008–2009, according to a common questionnaire. In total, 3501 questionnaires were collected, and the adherence with the recommendations of the Centre for Disease Control and Prevention (CDC) was evaluated.[2,10] Notification of 99 cases of neonatal GBS infections was received in the period from June 2007 to December 2010. The form included the collection of both maternal data (antenatal colonization, gestation at delivery, ethnicity, mode of delivery, risk factors and IAP) and neonatal data (sex, ethnicity, onset of symptoms, clinical manifestation, outcome and site of GBS isolation).

Neonatal GBS infections included only culture-proven cases. Sepsis, septic shock and bacteremia were identified according to conventional case definitions.[21]

Years 2012–2014 Surveillance

The passive surveillance was extended to any available national birth center. Forty-three reports on neonatal GBS infections were collected in 2012, 29 reports in 2013 and 23 reports in 2014. Only twenty-four bacterial strains were received.

Microbiologic Characterization

During the multicenter study, 91 GBS strains from NIs plus 320 GBS strains from the antenatal screening were collected and fully characterized. The 24 GBS strains received in the years 2012–2014 were only serotyped. Serotype analysis, surface protein markers determination, clonal relatedness and antibiotic resistance were performed as already described.[22–25]

Informed Consent

All data from the questionnaires were collected after patients, informed on the design of the study, gave their consent to participate.

Statistical Analysis

The Fisher exact probability test and the Wilcoxon–Mann–Whitney test were used to evaluate the categorical and continuous variables between groups, respectively. Two-sided P values <0.05 were considered statistically significant (SPSS 17 for Windows, IBM).

RESULTS

Clinical Features

Table 1 shows the data elaborated from the 3501 questionnaires, filled in at the time of delivery by the participating birth centers. The vaginal-rectal screening had a very high compliance (96.4%), and maternal GBS colonization rate (21.7%) was consistent with the reported carriage rate in Western Europe and Italy.[26–28]

The administration of IAP was variably consistent with CDC recommendations between birth centers, ranging from 37.5% to 100% (Table 2). Because of the lack of a national consensus guideline, IAP was also administered, in variable proportion, to women without indication for IAP (0.5%–31.5% of cases). Broad-spectrum antibiotics in combination with penicillin were administered in most of cesarean sections as part of infection-prevention protocols; however, some unnecessary IAP administrations could not be explained.

Characteristics of GBS NIs

On the whole, 95 cases of EOD and 99 cases of LOD were reported. The most frequent clinical manifestation of neonatal GBS infection was sepsis (51.5%) followed by bacteremia (15.5%), sepsis plus meningitis (12.4%), meningitis (7.7%) and septic shock (7.2%). Other minor clinical manifestations were septic arthritis (2.0%) and cellulitis (1.5%). Most neonates had full recovery (72.1%) but 11.3% had permanent sequelae; mortality was 5.1%.

TABLE 1. Characteristics of Deliveries

Median maternal age, yr	32 (15–48)
Type of delivery, %	
Vaginal	70.7
Planned caesarian section	19.3
Emergency caesarian section	10.0
Median gestational age, wk	39 (24–44)
Twin delivery, %	2.1
Risk factors, %	Yes, 11.8; No, 86.5; Not reported, 1.7
1	96.8
≥ 2	3.2
Risk factors description and prevalence, %	
Previous infant with invasive disease	1.5
GBS bacteriuria in the currency pregnancy	20.4
Delivery at < 37 wk gestation	28.0
Amniotic membrane rupture ≥ 18 h	45.0
Intrapartum temperature ≥ 38.0°C	4.4
Other	0.7
Antenatal GBS screening, %	96.4
Vaginal	8.2
Both vaginal and rectal	86.5
Not reported	5.2
Positivity	21.7
IAP, %	Yes, 29.0; No, 68.2; Not reported, 2.8

About 30% of neonatal GBS infections affected preterm babies, particularly preterm babies born before 35 gestation week, which coincides with the recommended time for the GBS antenatal screening. The maternal GBS status was known at labor in 70.6% of cases but it was negative in 34.7% of mothers whose babies subsequently developed EOD.

The characteristics of the 95 EOD cases are reported in Table 3. Emergency cesarean section was the most frequent mode of delivery in preterm neonates (20 out of 32 deliveries) and also the primary cause for missed IAP (23 out of 32 cases). Surprisingly, 9 out of 26 mothers had undergone the antenatal GBS screening outside the recommended gestational age (before 35 weeks gestation). Besides prematurity, additional risk factors were more frequently present in preterm births than full-term births. The majority of full-term neonates who developed EOD were born by vaginal delivery (69.8%); 81% of their mothers had performed the antenatal screening for GBS carriage that was negative in 29/63 (46.0%) of cases. Deviations from CDC indications were also observed: the presence of risk factors was considered an indication for IAP administration even if the GBS antenatal screening was negative. In almost all EOD cases in which IAP was administered (26 out of 30 cases), its duration was less than 4 hours.

The analysis of the characteristics of the 99 LOD cases, reported in Table 4, indicated that multiple births were more frequent than in EOD cases (11.1% vs. 4.2%, respectively) and they regarded also full-term neonates. The mean age of the onset of symptoms was higher in preterm neonates than in full-term neonates, particularly for those born before 35 weeks gestation (37.7 vs. 30.5 and 30.4 days, respectively; $P = 0.02$). Even if not prognostic for LOD, maternal GBS status and IAP administration were investigated. Most mothers of full-term neonates affected by LOD were screened negative for GBS carriage [37 out of 60 (61.6%)] and did not present risk factors at delivery [54 out of 65 (83%)]. IAP duration was more than 4 hours in 10.1% of LOD cases.

TABLE 2. Prevention Strategies Adopted at Labor Among Centers

Center	Women Eligible to IAP	IAP	Missing Information	Adherence to CDC, %	Women not Eligible to IAP	IAP	Missing Information	Total
Center 1	3	3	0	Not done	58	0	0	61
Center 2	196	188	0	95.9	618	195	0	814
Center 3	118	89	0	75.4	375	37	5	493
Center 4	8	3	0	37.5	68	14	0	76
Center 5	92	92	0	100	400	86	1	492
Center 6	122	50	0	41	380	15	58	502
Center 7	20	4	13	Not done	117	Not available	78	137
Center 8	131	99	0	75.6	306	44	0	437
Center 9	121	90	0	74.4	368	2	2	489
Total	811	618	13	76.2	2690	393	144	3501

TABLE 3. Characteristics of EOD (95 Cases)

Characteristics	Preterm Neonates (< 37 wk Gestation) (Total, 32)	Term Neonates (Total, 63)
Delivery		
Vaginal	12	44
Emergency cesarean section	20	13
Planned cesarean section	--	3
Not reported	--	3
Multiple births	4	--
GBS antenatal screening		
Done	15	51
Not done	17	11
Not reported	--	1
Positive	11	22
Negative	4	29
Risk factors		
1	21	13
> 1	11	5
Absent	--	43
Not reported	--	2
IAP		
Administrated	11	19
Not administrated	21	44
Duration > 4 h	1	3
CDC indications but notadministrated	18	12
	Emergency cesarean section (14) Precipitous delivery (1) Not reported (6)	Precipitous delivery (3) Home birth (1) Allergy (1) Emergency cesarean section (1) Not reported (6)
CDC nonindications butadministrated		Negative screening but risk factors (6)

TABLE 4. Characteristics of LOD (99 Cases)

Characteristics	Preterm Neonates (< 37 wk Gestation) (Total, 34)	Full-Term Neonates (Total, 65)
Delivery		
Vaginal	10	37
Emergency cesarean section	10	9
Planned cesarean section	14	15
Not reported	-	4
Multiple birth	8	3
GBS antenatal screening		
Done	12	60
Not done	22	3
Not reported	-	2
Positive	3	23
Negative	9	37
Risk factors		
1	26	9
> 1	6	None
Absent	2	54
Not reported	-	2
IAP		
Administered	10	25
Not administered	24	37
Not reported	-	3
Duration > 4 h	2	8
CDC indications but not administered	20	2
	Urgent caesarian section (17) Not reported (2) Precipitous delivery (1)	Not reported (2)
CDC nonindications but administered	-	5 Negative screening but risk factors (3) Not reported (2)

Serotype Distribution Among Neonatal and Maternal GBS Populations

During the multicenter study, serotype III was the most frequent (45%), almost 2-fold significantly enriched in NIs than in GBS-carrier PW (69.2% vs. 38.1%; $P = 0.00013$). On the contrary, serotypes Ia, II and V were significantly enriched in PW (17.8% vs. 8.8% for serotype Ia, $P = 0.03$; 12.5% vs. 4.4% for serotype II, $P = 0.03$; 20.6% vs. 9.9% for serotype V, $P = 0.01$). Other less-frequent serotypes were Ib (2.2% NI and 5.9% PW), IV (1.1% NI and 2.5% PW) and IX (3.3% NI and 1.6% PW). No serotypes VII and VIII were identified, and only one serotype VI strain was identified in PW (Fig. 1A).

Three GBS strains (1 isolated from LOD and 2 from PW) were not typable by either serologic or molecular typing methods (Table, Supplemental Digital Content 1, http://links.lww.com/INF/C598); further analysis demonstrated that two strains had a partial deletion of the *cps* locus and the other was a natural capsular mutant, which lacked the entire capsular locus, possibly by a recombination event.[29]

On the other hand, 87.7% of LOD cases were caused by the serotype III (Fig. 1B). This aspect was also confirmed by the serotyping of the 24 GBS strains collected in the years 2012–2014. The eight EOD strains belonged to serotypes Ia (2 strains), II (1 strain), III (4 strains) and V (1 strains). Fifteen out of the 16 GBS strains isolated from LOD were serotype III (the left one was serotype Ia).

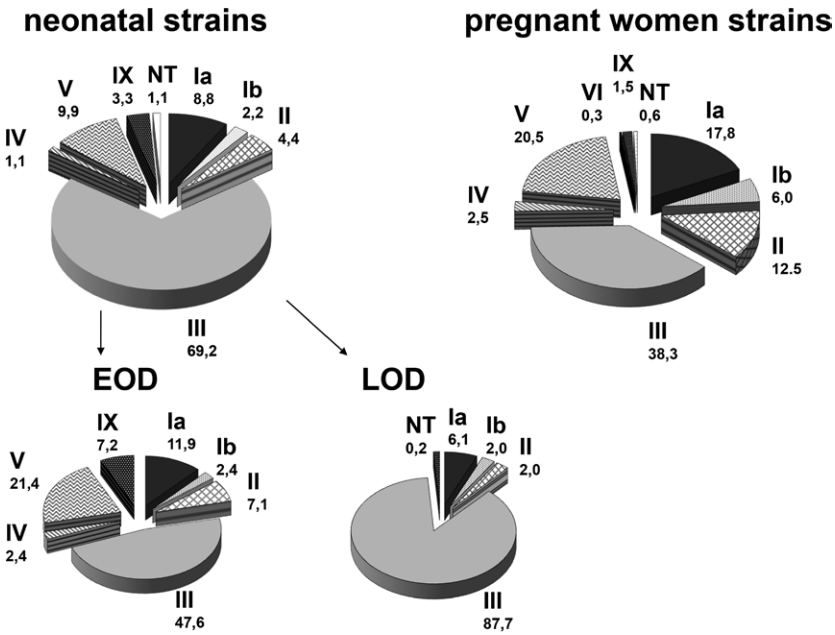

FIGURE 1. Serotype distribution in GBS strains isolated both from invasive neonatal infections and pregnant women. The numbers below the serotypes indicate the percent frequency. The prevalence of serotype III in GBS-colonized pregnant women (38.1%) differed significantly from the prevalence in LOD (87.7%; $P < 0.0001$) but did not differ from the prevalence in EOD (47.6%; $P = 0.244$).

MultiLocus Sequence Typing (MLST) and Clonal Analysis

MLST analysis identified a total of 43 sequence types (STs), of which 12 were new STs identified in this study. Maternal strains (126 MLST typed) displayed 35 STs, and neonatal strains (48 MLST typed) displayed 17 STs. Although neonatal and maternal strains could be grouped in the same 6 CCs (CC-1, CC-12, CC-17, CC-19, CC-23 and CC-24), only 9 STs were shared between neonatal and maternal strains (Table 5).

Serotype and surface protein genes were largely predictive of CC (Table, Supplemental Digital Content 1, http://links.lww.com/INF/C598). Serotype Ia was prevalently associated to either CC-23 possessing alp1 or CC-24 possessing alpha-C; serotype Ib was associated to CC-12 and alpha-C; serotype II was associated to CC-12 (either alpha-C or alp2/3) and CC-19 (possessing rib); serotype III was mostly associated to CC-17 and to a lesser extent to CC-19 (both possessing rib); serotype IV was associated to CC-1 and alp1; serotype V was prevalently associated to CC-1 and alpha-C and serotype IX strains belonged to the singleton ST130 (either alpha-C or alp2/3). Four serotype V/ST26 strains were isolated from 2 EOD cases and 2 antenatal swabs. Putative capsular switching events were also observed; 1 serotype IV/ST291 strain belonged to CC-17, the most diffuse clone among NIs, associated to the serotype III.

TABLE 5. Description of STs in Neonatal and Maternal Strains

Source	ST Types
Neonatal strains (48/91)	**1 (3)**, 6 (1), **10 (1)**, **12 (1)**, **17 (26)**, **19 (2)**, **23 (1)**, **26 (2)**, **28 (1)**,**130 (3)**, **196 (1)**, 420 (1), 467 (1), 496 (1), 498 (1), 529 (1), 555 (1)
Maternal strains (126/320)	**1 (16)**, 2 (4), 7 (2), 8 (4), **10 (3)**, **12 (5)**, **17 (24)**, **19 (6)**, 22 (2), **23 (17)**, 24 (2), **26 (2)**, **28 (8)**, 88 (2), 110 (1), **130 (4)**, 136 (1), 144 (1), 180 (1), 188 (1), **196 (5)**, 248 (1), 291 (1), 328 (2), 452 (1), 459 (1), 487 (1), 531 (1), 533 (1), 534 (1), 535 (1), 556 (1), 560 (1), 561 (1), 562 (1)

Numbers in bold are the STs shared between neonatal and maternal strains. Numbers in parenthesis indicate the total of typed strains.

Erythromycin and Clindamycin Resistance

Erythromycin resistance was detected in 17.0% of strains (15.4% and 17.5% of NI and PW strains, respectively). Resistance to clindamycin was slightly lower than 15.3% (13.2% and 15.9% of NI and PW strains, respectively) because only 6.7% of strains displayed the M phenotype. The constitutive high level of resistance to both erythromycin and clindamycin (phenotype CR) was possessed by 71.4% of resistant strains (mediated mostly by the *erm*B gene), and the inducible resistance to clindamycin (phenotype IR) was displayed by 21.4% of resistant strains (mediated by the *erm*A gene) (Table, Supplemental Digital Content 1, http://links.lww.com/INF/C598). Some atypical phenotypes/genotypes were observed: 2 strains that displayed constitutive resistance to erythromycin and clindamycin were negative to all the *erm* and *mef* genes tested; 2 strains with inducible resistance possessed the rare *erm*(T) gene determinant and 2 strains displayed clindamycin resistance and erythromycin sensitivity (CRES phenotype; Table, Supplemental Digital Content 1, http://links.lww.com/INF/C598) for which the genetic determinant could not be identified.

DISCUSSION

The need of national recommendations has been reinforced by this study that demonstrated how the GBS prevention strategies varied between participating centers, despite the effort of some area-based networks to use common consensus protocols.[22,30–32] Indeed, the advice to use a vaginal-rectal GBS screening at the third trimester of pregnancy and IAP administration to carriers has been recently included (in the year 2011) in a national guideline on the management of uncomplicated pregnancy; nevertheless, specific national guidelines on the neonatal GBS disease prevention are still lacking. The study highlighted the importance of an active surveillance system: the second period of study witnessed a gradual decline in the number of reported cases of infections (50 cases in 2012, 33 cases in 2013 and 25 cases in 2014), and it would be advisable to investigate if this finding, more than reflecting a real reduction of neonatal GBS disease, was an indication that the voluntary participation decreased with time.

Provided the intrinsic limitations to extrapolate data from a multicenter study to a national level, the evaluation of the neonatal

GBS disease risk at delivery comprising birth centers evenly geographically distributed was never investigated before, and the present data still remain the most representative picture of the prevention policies adopted by hospitals in our country.

In early studies on neonatal GBS disease, EOD exceeded of about 2–3-folds the LOD burden, representing approximately 75% of all GBS cases.[2] At present, EOD equals LOD cases,[10,12,17] a trend confirmed by this study where the EOD incidence rate was comparable to LOD during the whole time period. Moreover, 94.8% of EOD cases had their onset in the first 48 hours of life, of which 52.1 % were sick at birth.

Preterm deliveries, precipitous labor and GBS-negative screened mothers represent, nowadays, the prominent failures of the prevention strategies. Indeed, one-third of GBS infections regarded preterm infants, 80% of which were born before 35 weeks of gestation. Missed or inadequate IAP was more frequent in EOD affecting preterm (70%) than full-term neonates (53.6%) because of the precipitous delivery that often characterizes such births. Other less frequent missed opportunities for IAP included an inadequate route of antibiotic administration, the use of macrolides to which the GBS resulted resistant (2 EOD cases), home birth.

About 35%–40% of EOD are from GBS-negative screened mothers.[2,12] The recommended combined rectal-vaginal swabbing increased sensitivity,[10] but the relevant proportion of perinatal infections from negative mothers strongly suggests that the real adherence to the specimen processing protocol should be evaluated and, in case, new solutions should be proposed. The introduction of both chromogenic media and rapid molecular diagnostic assays to be used at the time of delivery has been proposed[10,33,34]; nevertheless, it cannot be predicted whether the use of such methods would further reduce the incidence of EOD.

Regardless of the presence of intrapartum risk factors, CDC guidelines do not recommend IAP if women in labor are GBS screened negative.[10] This approach was rarely followed by the participating birth centers, which preferred to administrate IAP in this situation, representing the most common deviation from the CDC guidelines.

In the case of LOD, the relevant proportion of mothers who had received IAP confirmed its inefficacy in disease prevention and the need of a better understanding of the bacterial transmission mode. Indeed, while the serotype distribution of GBS strains isolated from both EOD and vaginal-rectal antenatal screening was very similar, confirming that the vertical transmission from mother to baby is the principle cause of disease, the majority of LOD cases was caused by serotype III strains belonging to CC-17, a clonal lineage which is worldwide diffused.[17] The reason for its success in promoting invasive disease and preponderance in LOD is still modestly defined but it is presumably correlated to peculiar virulence factors conferring an higher ability to both survive to phagocytic killing and to translocate the blood-brain barrier.[35]

Major efforts to understand the dynamics of both neonate and mother GBS colonization along with the characterization of the GBS clones in the postpartum temporal window critical for LOD onset would be advisable. The use of IAP clearly decreases the density of the colonizing GBS, but it does not influence the maternal GBS colonization status[36] and most GBS-positive PW are again stably colonized 8 weeks postpartum.[37] Newborns exposed to IAP and GBS-free at the time of the hospital discharge subsequently acquired GBS from their mothers.[37,38]

Epidemiologic studies revealed a very stable clonal structure of GBS, as reported in different countries.[9,18] Nevertheless, peculiar enrichments within the GBS neonatal and maternal populations were observed in our study.

A striking finding was the capsular switching from serotype III to IV that occurred within the very homogeneous CC-17 in 1 ST291 maternal strain, already reported and considered an alert for the possible immune escape of this hypervirulent clone once a vaccine is available.[39] However, even if an increase of serotype IV strains has been reported in US, Canada and Europe[40–42] reaching up to 14% of incidence among pregnant carrier women in Norway,[27] the frequency of ST291 within serotype IV strains is very low and stable in time. Because the serotype IV-ST291 clone was first isolated in US from a collection dating back the late 90s,[40] very few reports have been published.[39,41,43]

The erythromycin resistance rate in neonatal strains (15%) was slightly higher than that reported in our previous area-based survey (12%),[25] but it was lower than the resistance rate observed in maternal strains (17.5%). The higher rate of erythromycin resistance in PW strains was due to the higher proportion of the serotype V/CC-1 clone, whose association with erythromycin resistance (carried by the *erm*B gene) has been reported since more than 10 years.[25] The low prevalence of the M phenotype support the CDC recommendations that, if the GBS isolate is erythromycin resistant and clindamycin sensitive, the possibility of the inducible resistance to clindamycin should be always tested in the case of IAP administration of penicillin-allergic women.

This rate was similar to the rate of erythromycin resistance in adults (16.5%) in our previous report conducted in the years 2002–2005[44] indicating that no relevant fluctuations over time have occurred in our country. Nevertheless, rare and atypical phenotypes (CRES phenotype) whose genetic determinants are still unknown have been detected, and their frequency in the GBS population should be monitored over time.

This study demonstrated that measures for reducing prevention failures and EOD incidence by a higher adherence to the CDC recommendations and, possibly, by the issue of a national guideline have to be encouraged. The administration of IAP to negatively GBS-colonized women in presence of prolonged rupture of membranes and/or fever has been one cause of deviation from the CDC protocol, and it should deserve further considerations.

Nevertheless, because even perfect adherence to the recommended prevention guidelines would not completely prevent neonatal GBS disease[2] and, despite efforts, a still not negligible proportion of permanent neurologic sequelae and mortality are present, vaccination of PW is an important desirable strategy to ensure that protective levels of antibodies are present at delivery.[45,46]

A trivalent conjugate vaccine (serotypes Ia, Ib and III) is currently being tested on healthy PW[47,48]; it will possibly reduce not only EOD burden but also that of the preterm GBS-related births and LOD.

In view of the above considerations, this prevaccine era urges the importance of the maintenance of surveillance systems to monitor the impact of future vaccines and to support effective strategies for the neonatal GBS disease prevention.

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