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Serotypes, Genotypes, And Antibiotic Susceptibility Profiles Of Group B Streptococci Causing Neonatal Sepsis And Meningitis Before And After Introduction Of Antibiotic Prophylaxis

Trijbels-Smeulders, Monique A. J. M. MD*†; Kimpen, Jan L. L. MD‡; Kollée, Louis A. A. MD*; Bakkers, Judith MD§; Melchers, Willem PhD§; Spanjaard, Lodewijk MD[//]; Wannet, Wim J. B. PhD¶; Hoogkamp-Korstanje, Mieke A. A. MD§

From the *Department of Paediatrics, University Medical Centre (UMC ST Radboud), Nijmegen, The Netherlands; †Department of Paediatrics, Flevoziekenhuis, Almere, The Netherlands; ‡Department of Paediatrics, Wilhelmina Children's Hospital, University Medical Centre, Utrecht, The Netherlands; §Department of Medical Microbiology, University Medical Centre (UMC ST Radboud), Nijmegen, The Netherlands; [//]Netherlands Reference Laboratory for Bacterial Meningitis, Academic Medical Center, Amsterdam, The Netherlands; and the ¶National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands.

Address for correspondence: Monique A. J. M. Trijbels-Smeulders, Department of Paediatrics, University Medical Centre UMC St Radboud, PO Box 9101, 6500 HB Nijmegen, The Netherlands. E-mail Trijbels@wxs.nl.

Abstract:

We studied the characteristics of strains isolated from neonates with group B streptococci sepsis and meningitis, before and after the introduction of antibiotic prophylaxis in The Netherlands. In 1999, 1 year after this introduction the serotype and genotype distribution and the susceptibility patterns of the GBS strains had not changed. Penicillins remain drugs of first choice to prevent and treat neonatal GBS disease.

In the Netherlands, nationwide guidelines for prevention of early-onset GBS infection were introduced in January 1999. Thereafter, we found a decline of the incidence of early-onset GBS sepsis from 0.54 per 1000 live births in 1997–1998 to 0.36 per 1000 live births in 1999–2001. Intrapartum administration of antibiotics increased from 1% to 5.9%; amoxicillin/clavulanic acid was used mostly and penicillin was hardly used.1

During the past 25 years, the case fatality rate of neonatal early-onset GBS sepsis has decreased from about 55% to 5%–10%, partly due to improved neonatal care,2 but changes in the characteristics of the microorganism might have contributed as well.

In this study, we typed GBS-strains isolated in the Netherlands in 1997–1999 from patients with neonatal sepsis and meningitis and we studied the relationship between the clinical presentation of the disease and the serotype and/or genotype of GBS strains isolated before (1997–1998) and after introduction of antibiotic prophylaxis (1999). Finally, we determined the resistance patterns of the GBS-strains isolated before and after introduction of antibiotic prophylaxis to trace emergence of resistance and to evaluate whether the current antimicrobial approach in neonatal GBS disease in The Netherlands is still adequate.

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MATERIALS AND METHODS

Clinical Data.

Basic clinical data of 198 neonates hospitalized for GBS sepsis and/or meningitis in the Netherlands were obtained during the period 1997–1999. Additional clinical information for 108 of them was available because they were included in an epidemiologic study.1

Strains.

We studied 119 GBS strains in 1997–1998 and 79 GBS strains in 1999 isolated from blood (N = 147), CSF (N = 20), and both (N = 31) of neonates with GBS disease hospitalized in 51 out of the 93 neonatal and pediatric wards in the Netherlands. The strains were obtained from 22 Laboratories for Medical Microbiology and from the Netherlands Reference Laboratory for Bacterial Meningitis in the Academic Medical Centre Amsterdam.

Serology.

Serogrouping was carried out by the Streptex method. Serotyping was performed with the agglutination test using monospecific antisera against GBS serotypes Ia, Ib, II, III, IV, V, VI, VII, VIII.

Genotyping.

Isolates were stored in Todd-Hewitt broth with 5% defibrinated sheep blood at -80°C until used for genetic characterization. For pulsed-field gel electrophoresis (PFGE), the isolates were treated essentially as described by Benson and Ferrieri.3 The chromosomal DNA was digested with 50 U *Sma*I for 3 hours at 25°C, and the DNA was separated with a contour-clamped homogeneous electric field (CHEF DR-II, Bio-Rad Laboratories, Richmond, CA). [lambda] Phage concatemers were run simultaneously as a size marker. Pulse times were ramped from 2.2 to 54.2 seconds over 24 hours at 200 V and 14°C. The gels were stained with ethidium bromide, and PFGE patterns were compared visually.4

Controls were 2 epidemiologically unrelated GBS strains (the NCTC-8181 T strain and a strain obtained from the National Institute of Public Health and the Environment).

Susceptibility Testing.

MICs were determined in duplicate by broth microdilution with IsoSensitest Broth (Oxoid CM 491, Haarlem, the Netherlands) supplemented with 2% lysed horse blood for fastidious microorganisms. The inoculum was prepared by the direct colony suspension method: colonies grown overnight on Columbia agar with 5% sheep blood were suspended in sterile saline up to a concentration of 0.5 McFarland turbidity standard. After dilution, the final inoculum was 4×10^5 cfu/mL. The antimicrobial agents tested were penicillin, amoxicillin, ceftazidime, cefixime, cefepime, meropenem, erythromycin, clarithromycin, vancomycin, teicoplanin, quinupristin-dalfopristin, ciprofloxacin, trovafloxacin and moxifloxacin. The antibacterial agents were obtained from the original manufacturers, and stock solutions were prepared following the manufacturer's instructions. Concentrations ranged from 0.03 to 64 mg/L for all drugs. The trays were incubated at 37°C and examined for growth after 24 hours. The breakpoints

were defined by the Clinical and Laboratory Standards Institute (CLSI)5; when breakpoints were unknown, breakpoints for comparable drugs or comparable microorganisms were taken. Control strains were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212.

Pk/Pd Analyses.

Pharmacodynamic principles were used to predict the usefulness of an antibiotic for treatment of GBS meningitis. We calculated the concentrations of the test drugs in serum and CSF during 24 hours after standard dosing schedules for neonates,6 assuming that the CSF half-life time is comparable with serum half-life time.7 Further we determined the time during which the concentrations would exceed 10 times the MIC, being the appropriate pharmaco-dynamic figure for [beta]-lactams 8 for optimal killing in experimental Gram-positive meningitis.9 The areas under the curve during 24 hours (AUC24) in CSF were calculated for quinolones,10–12 and the ratio between AUC24 and the MIC was determined, being the appropriate pharmacodynamic parameter for quinolones.13

RESULTS

The 198 neonates with GBS sepsis and/or meningitis included 105 (53%) males. Additional information was obtained from 108 neonates, of these 26% (28/108) were preterm (<37 weeks) and the case-fatality rate was 7% (8/108). The GBS strain serotype Ia was isolated in 4 of the 8 patients who died.

We were able to serotype 184 (93%) of the 198 strains. Serotypes Ia (37 [19%]) and III (109 [55%]) predominated and serotypes VII and VIII were not found. The relationship between serotype and clinical manifestation of the disease is presented in Table 1.

TABLE 1. Clinic Manifestation and Serotypes of 198 GBS Strains From Neonates With GBS Sepsis and/or Meningitis in 1997–1999 in the Netherlands

Clinic	Total Number															
	N	%	Ia	%	Ib	%	II	%	III	%	V	%	Other	%	Nontypable	%
Early-onset (≤7 d)	142	72														
Sepsis	113	57	24	12	5	2.5	15	7.5	46	23	12	6	1	0.5	10	5
Meningitis	5	3	2	1	—	—	—	—	2	1	1	0.5	—	—	—	—
Sepsis + meningitis	24	12	4	2	—	—	—	—	20	10	—	—	—	—	—	—
Late-onset (>7 d)	56	28														
Sepsis	22	11	3	2	1	0.5	1	0.5	14	7	—	—			3	1.5
Meningitis	10	5	2	1	—	—	—	—	5	2.5	1	0.5	1	0.5	1	0.5
Sepsis + meningitis	24	12	2	1	—	—	—	—	22	11	—	—	—	—	—	—
Total	198	100	37	19	6	3	16	8	109	55	14	7	2	1	14	7

There was no difference in the serotype distribution between the 2 periods (1997–1998 and 1999).

The GBS strains were genetically heterologous, 198 distinct PFGE patterns were found, and there was no difference found between the 2 periods.

For all antimicrobial agents we tested, the MIC distributions were unimodal and the MIC90 varied from <=0.03 to 1.00 mg/L. Forty-five percent of the GBS-strains was intermediate susceptible to ciprofloxacin (MIC 90 = 1 mg/L), whereas the GBS-strains were susceptible to all other agents.

The concentrations of penicillin, amoxicillin, cefixime, and cefepime remained above the required concentration during the entire period. The concentration of

ceftazidime was adequate during 6 hours after the first dose and again after the second dose. The concentration of meropenem is lower than 10 times MIC 90 of GBS from 9–12 hours and from 21–24 hours, using the dosing schedule with 2 gifts. The concentrations in CSF and the areas under the curves during 24 hours (AUC24), calculated for the quinolones are presented in Table 2. The ratios between AUC24 in CSF and MIC 90 were 9.9 for ciprofloxacin, 74.8 for trovafloxacin and 59.6 for moxifloxacin. The figure for ciprofloxacin was below the required ratio of 30 for effective killing; those for trovafloxacin and moxifloxacin were well above that basis.

TABLE 2. Pk/Pd Analysis for Quinolones in CSF

Drug	T1/2 (h)	Dose (mg/kg)	Dose Interval (h)	Serum Concentration (mg/L), T1	CSF Concentration (mg/L)				24-h AUC (mg/L/h)	MIC_{90}	Pk/Pd
					T1	T4	T8	T12			
Ciprofloxacin	4	7.5	2	3	0.8	0.5	0.25	0.13	9.9	1	9.9
Trovafloxacin	10	7.5	1	6	1.5	1.27	0.97	0.7	18.7	0.25	74.8
Moxifloxacin	10	7.5	1	5	1.2	1.02	0.78	0.57	14.9	0.25	59.6

DISCUSSION

In the Netherlands, the serotype distribution of GBS strains did not change during the first year (1999) after the introduction of antibiotic prophylaxis. Maybe this period of 1 year is too short to identify changes, and therefore we compared our serotype distribution in 1998–1999 with the serotype distribution that was found in a study on GBS epidemiology in the Netherlands in 1980.14 As in 1980, serotype III was found frequently in 1997–1999. In 1980, serotype Ib predominated, and in 1997–1999 serotype Ia was more frequently found. A possible explanation for this difference is that the serotyping was performed in a different group of neonates. In 1980, the serotyping was performed in healthy neonates of mothers who were GBS carriers, and in 1997–1999, in neonates with early-onset GBS sepsis and meningitis. Serotypes Ia and III were predominant in patients with early-onset GBS sepsis, and serotype III, in patients with late-onset GBS sepsis and/or meningitis. This association of serotypes with the type of clinical disease was found by others, as well.15–17

The GBS strains were genetically heterologous; 198 distinct PFGE patterns were found. These findings confirmed a previous study where we observed that the strains from 10 mothers–GBS carriers were genetically different, but the strains from each mother and her own child were identical.18 Hansen et al 19 found the same results in GBS carriers.

We could not demonstrate any shift in susceptibility after the introduction of penicillin or amoxicillin prophylaxis for prevention of neonatal GBS disease; the same result was found by Fernandez et al.17 Because all strains were highly susceptible to all [beta]-lactams tested, they all may be effective for treatment of GBS septicemia. Not all [beta]-lactams showed equal pharmacodynamics: the concentrations of ceftazidime and meropenem were not continuously maintained above the 10 × MIC using the standard dosing schedules. We think that the traditional penicillins are preferable to cephalosporins and meropenem for treatment of GBS meningitis and sepsis.

Teicoplanin was the most active glycopeptide. This antibiotic drug is not the glycopeptide of first choice in most neonatal units, but because of its activity and the dosing of once daily, it might be considered as an alternative for septicemia when [beta]-lactams are contraindicated. Because of poor diffusion into the cerebrospinal fluid, even in case of meningitis, neither vancomycin nor teicoplanin is suitable for treatment of GBS meningitis. This is also true for quinupristin and

dalfopristin, the activity of which is inferior to that of vancomycin and teicoplanin. Experience with quinupristin and dalfopristin in the treatment of neonatal disease is limited.

Erythromycin and clarithromycin showed equal antimicrobial activity, but the experience with macrolides for treatment of GBS disease is limited, and because of poor penetration into the cerebrospinal fluid, they are not suitable for treatment of GBS meningitis.

Quinolones are seldom used in neonates. There are few data on susceptibility of GBS to quinolones, especially the new quinolone moxifloxacin, which is advertised as more active against Gram-positive cocci than ciprofloxacin. It is good to know how active moxifloxacin is against GBS. There are only few data on quinolones in Gram-negative meningitis.

In conclusion, 1 year after the introduction of antibiotic prophylaxis in the Netherlands, the serotype and genotype distribution and the susceptibility patterns of the GBS strains did not change, and penicillins remain drugs of first choice to prevent and treat neonatal GBS disease.

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REFERENCES

1. Trijbels-Smeulders M. Group B streptococcal disease: effect of the dutch guidelines for prevention. Thesis. Radboud University Nijmegen. 2006;ISBN:90-9020576-4.

2. Schrag SJ, Zywicki S, Farley MM, et al. Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. *N Engl J Med*. 2000;342:15–20.

3. Benson JA, Ferrieri P. Rapid pulsed-field gel electrophoresis method for Group B streptococcus isolates. *J Clin Microbiol*. 2001;39:3006–3008.

4. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol*. 1995;33:2233–2239.

5. National Committee for Clinical Laboratory Standards. *Performance Standards for Antimicrobial Susceptibility Testing: Eleventh Informational Supplement M110-S11, NCCLS 2001*. Wayne, PA: National Committee for Clinical Laboratory Standards.

6. Hartwig NG, Kornelisse RF, Verduin CM. *Blauwdruk Pediatrische Antimicrobiele Therapie.* 2001.

7. Reed MD, Goldfarb J, Blumer JL. Anti-infective therapy. In: Jenson HB, Baltimore RS, eds. *Pediatric Infectious Diseases: Principles and Practice*. Norwalk, CT: Appleton and Lange; 1995.

8. Lutsar I, McCracken GH, Friedland IR. Antibiotic pharmacodynamics in cerebrospinal fluid. *Clin Infect Dis*. 1998;27:1117–1129.

9. Tauber MG, Doroshow CA, Hackbarth CJ, et al. Antibacterial activity of beta-lactam antibiotics in experimental meningitis due to *Streptococcus pneumoniae*. *J Infect Dis*. 1984;149:568–574.

10. Cutler NR, Vincent J, Jhee SS. Penetration of trovafloxacin into cerebrospinal fluid following intravenous infusion of alatrofloxacin. *Antimicrob Agents Chemother*. 1997;41:1298–1300.

11. Isaacs D, Slack MPE, Wilkinson AR. Successful treatment of *Pseudomonas ventriculitis* with ciprofloxacin. *J Antimicrob Chemother*. 1986;17:535–538.

12. Owens RC, Ambrose PG. Pharmacodynamics of quinolones. In: Nightingale CH, Murakawa T, Ambrose PG, eds. *Antimicrobial Pharmacodynamics in Theory and Clinical Practice*. New York, NY: Marcel Dekker; 2002.

13. Ambrose PG, Grasela DM, Grasela TH, et al. Pharmacodynamics of fluoroquinolones against *Streptococcus pneumoniae*: analysis of phase-III clinical trials. *J Antimicrob Chemother*. 2001;45:2793–2797.

14. Gerards LJ, Cats BP, Hoogkamp-Korstanje JAA. The influence of group B streptococcal-carriership on pregnancy outcome. *J Perinat Med*. 1982;10:279–284.

15. Baker CJ. Group B streptococcal infections. *Clin Perinatol*. 1997;24:59–70.

16. Harrison LH, Elliot JA, Dwyer DM, et al. Serotype distribution of invasive group B streptococcal isolates in Maryland: implications for vaccine formulation: Maryland Emerging Infection Program. *Infect Dis*. 1998;177:998–1002.

17. Fernandez M, Hickman ME, Baker CJ. Antimicrobial susceptibilities of group B streptococci isolated between 1994 and 1996 from patients with bacteremia or meningitis. *Antimicrob Agents Chemother*. 1998;42:1517–1519.

18. Melchers WJG, Bakkers JMJE, Toonen M, Kuppeveld FJM, Trijbels M, Hoogkamp-Korstanje JAA. Genetic analysis of *Streptococcus agalactiae* strains isolated from neonates and their mothers. *FEMS Immunol Med Microbiol*. 2003;36:111–113.

19. Hansen SM, Uldberg N, Kilian M, et al. Dynamics of *Streptococcus agalactiae* colonization in women during and after pregnancy and in their infants. *J Clin Microbiol*. 2004;42:83–89.