JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2003, p. 4688–4694 Vol. 41, No. 10
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.10.4688–4694.2003

Six-Month Multicenter Study on Invasive Infections Due to Group B Streptococci in Argentina

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Received 9 April 2003/Returned for modification 9 June 2003/Accepted 30 July 2003

There is little information about invasive infections by group B streptococci (GBS) and their antimicrobial susceptibilities in Latin America. We performed a prospective multicenter study to determine the serotype distribution and the antimicrobial susceptibility of GBS in Argentina. We identified 58 cases, but only 44 had sufficient data to be evaluated. Eight early-, four late-, and one fatal late, late-onset neonatal infections due to GBS were found. A total of 31 patients were adults with bacteremia, skin and soft tissue infections, osteomyelitis, arthritis, meningitis, abdominal infections, and renal abscess. Serotype III was prevalent in late-onset neonatal disease, and several serotypes (Ia/c, III, Ia, and II) were involved in early-onset neonatal infections. Serotypes II, Ia/c, III, and IV were commonly found in adults, with serotype II prevalent in younger adults (18 to 69 years old) and serotype Ia/c prevalent in elderly adults (>70 years old). The mortality rate attributable to GBS infections was 10.8%. All GBS were susceptible to penicillin and ceftriaxone. Resistance to clindamycin (1.7%), erythromycin (5.2%), azithromycin (5.2%), minocycline (69%), and tetracycline (72.4%), to high levels of kanamycin and amikacin (1.7%), and to intermediately high levels of gentamicin (1.7%) was observed. The bifunctional enzyme AAC6′-APH2″ was detected in the isolate resistant to aminoglycosides, and other genetic determinants were identified in other resistant isolates: *tetM* and *tetO* in tetracycline-resistant streptococci and *mefA* and *ermTR* for efflux-mediated and inducible macrolide-lincosamide-streptogramin B-resistant streptococci, respectively. For clinical purposes and rapid and easy detection of high-level aminoglycoside-resistant GBS, a screening method that used 1,000-μg kanamycin disks is proposed.

Streptococcus agalactiae or group B streptococcus (GBS) according to the Lancefield classification, is a recognized human pathogen that is mainly involved in perinatal disease despite effective prevention programs. Sepsis, pneumonia, urinary tract, skin, and soft tissue infections have been also reported, especially in elderly adults (44). New capsular serotypes have recently been described for GBS, and they were found to cause clinical infections (43).

During the last decade there has been an increased interest in invasive infections by GBS; however, there are still few data available from Latin America.

Penicillin (PEN) resistance has not yet been demonstrated in GBS. PEN tolerance, a phenomenon of questioned significance, was detected in GBS, including Argentinian strains (4, 21, 49). Time-kill curves showed the in vitro enhanced killing activity of beta-lactams when an aminoglycoside (AG) was added (49). Although PEN or ampicillin is the antibiotic of choice for GBS infections, the combined use of a beta-lactam plus an AG was suggested by Isaacs and Wilkinson for treating GBS neonatal sepsis, meningitis, and endocarditis (16). As described for enterococci, such a combination could achieve a bactericidal synergy in the absence of an enzymatic mechanism of resistance (29). In enterococci, the detection of high-level AG resistance can predict failure in the bactericidal synergy (42); however, nothing was established for GBS, although one highly gentamicin (GEN)-resistant strain and several highly streptomycin (STR)-resistant strains have been described (7, 28).

Erythromycin (ERY) is regarded as an alternative to PEN in the prophylaxis or treatment of infections due to GBS in PEN-allergic patients.

The most commonly described macrolide resistance mechanisms in gram-positive cocci are target site modification (50) and efflux (47). Target site modification is determined by the *erm* genes, which codify a methylase that leads to the inducible or constitutive expression of the MLS_B resistance phenotype (50). In the first instance, ERY and other 14- and 15-membered macrolides act as inducer agents, whereas clindamicin (CLI), streptogramins B, and 16-membered macrolides become inactive when inducers are present. The constitutive MLS resistance phenotype is characterized by resistance to all macrolides, lincosamides, and streptogramins B, even without the presence of an inducer (22). Phenotype M, due to active efflux, was described as mediated by the *mefA* (subgroups *mefA* or *mefE*) gene in GBS (2).

High rates of tetracycline (TET) and chloramphenicol resistance have also been found in GBS (14). TET resistance is due to acquisition of *tet* determinants that codify for antibiotic efflux or ribosomal protection in gram-positive cocci (40). In

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Staphylococcus, *Enterococcus*, and *Streptococcus* spp., efflux genes such as *tetK* and *tetL* (32, 35, 37) and ribosomal protection genes such as *tetM*, *tetO*, *tetT*, and *tetU* were the most commonly described determinants (8, 23, 51).

We sought here (i) to analyze the relationship between the serotype distribution and the clinical presentation of patients, (ii) to determine the susceptibility to 10 antimicrobial agents of the GBS isolates in a prospective study performed during a 6-month period (1 October 1998 to 31 March 1999) in 40 centers of 16 Argentinian cities, (iii) to identify the mechanisms involved in the antimicrobial resistance, and (iv) to propose a simple screening test for detecting highly AG-resistant GBS in the clinical laboratory.

MATERIALS AND METHODS

Clinical isolates and patients. All GBS strains isolated from invasive infections in the participating centers during the time period (1 October 1998 to 31 March 1999) were used for the present study. Not all cases in all geographical areas were obtained because not all of the centers participated in the study.

Samples were obtained from skin and soft tissues, bones, joints, blood, central nervous system, pleura, abdominal cavity, and kidney. All isolates were included for the antimicrobial susceptibility analysis, and all of them were also serotyped, but serotype distribution was determined only with strains isolated from patients whose age, sex, diagnosis (source), and underlying disease(s) were known.

Tests for identification and characterization. Hemolysis was detected in 5% sheep blood Columbia agar. L-pyrroglutanyl-aminopeptidase (PYR), leucine-aminopeptidase (LAP), and bacitracin tests were performed by using Britania disks (Britania, Buenos Aires, Argentina). Gram stain smears were prepared from bacteria grown in thioglycolate broth. The identification was completed by latex agglutination (Slidex Strepto-Kit; bioMérieux, Marcy l'Etoile, France) and the CAMP test.

Serotyping was performed by a capillary precipitin test. Antisera to polysaccharide antigens Ia, Ib, II, III, IV, V, VI, VII, and VIII and protein antigen C were used. Antisera were prepared at the Centers for Disease Control and Prevention, Atlanta, Ga., and at the Staten Seruminstitut, Copenhagen, Denmark.

Antimicrobial susceptibility tests. Disk diffusion tests were performed by the Kirby-Bauer method according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines by using 5% sheep blood Mueller-Hinton agar (30). Disks of PEN (10 U), ERY (15 μg), CLI (2 μg), and TET (30 μg) were from BBL (Becton Dickinson, Cockeysville, Md.). Plates were incubated at 35°C for 24 h in normal atmosphere. Blunting of the CLI inhibition zone near the ERY disk indicated an inducible type of MLS resistance, whereas no blunting indicated the probability of an efflux-mediated M phenotype (macrolide resistance phenotype) (46). Resistance to both ERY and CLI indicated a constitutive type of MLS resistance. Disks of GEN (120 μg), STR (300 μg), and kanamycin (KAN; 120 μg), currently used to detect AG high-level resistance in enterococci, were used as a possible screening method for predicting AG–beta-lactam synergy resistance in beta-hemolytic streptococci (42).

The E-test, performed as recommended by its manufacturer with strips of GEN (0.064 to 1,024 μg/ml), KAN (0.016 to 256 μg/ml), STR (0.064 to 1,024 μg/ml), and amikacin (AMK; 0.016 to 256 μg/ml) (AB Biodisk, Solna, Sweden), was used for recognizing high-level AG-resistant strains.

The agar dilution method according to NCCLS guidelines was used for susceptibility testing to seven antibiotics (31). Different concentration ranges were used for these drugs as follows: for PEN and ceftriaxone (CRO), 0.007 to 4.0 μg/ml; and for ERY, CLI, azithromycin (AZM), TET, and minocycline (MIN), 0.06 to 128 μg/ml. For category interpretation of MIN results, we used the breakpoints recommended for TET.

Staphylococcus aureus 29213, *Enterococcus faecalis* ATCC 51299, and *Enterococcus faecalis* ATCC 29213 were used as reference strains for the antimicrobial susceptibility tests.

Time-kill curves were determined by using appropriate concentrations of PEN, GEN, STR, and AMK, either alone or in combination (PEN concentrations were always above the MIC, and AG concentrations were always below the MIC) in a final volume of 5 ml of 2% lysed horse blood Mueller-Hinton broth. Aliquots of 0.15 ml were withdrawn from the vials at different times (0, 6, and 24 h); a 0.05-ml portion of each aliquot was spread with a glass rod on sheep blood agar plates, on which colony counting was performed after 48 h. The remaining volume (0.1 ml) was serially diluted (1/10), and then each dilution was spread in volumes of 0.05 ml on other sheep blood agar plates. A carryover effect was ruled out by inhibition tests over a confluent growth of *Staphylococcus aureus* ATCC 25923.

DNA extraction. Genomic DNA was extracted as described by Pitcher et al. (36).

PCR amplification. The presence of the *tetM*, *tetO*, *tetK*, *tetL*, *mefA*, *mefE*, *msrA*, *ermA*, *ermB*, *ermC*, *ermM*, and *ermTR* genes was determined by the standard PCR technique. All of the PCRs were carried out by denaturation for 10 min at 94°C, followed by 35 amplification cycles and a final elongation at 72°C with previously described primers (5, 33) or using the OLIGO software (see Table 1). For *tetM* (397 bp) detection, the cycles consisted of 1 min at 94°C, 1 min at 46°C, and 1 min at 72°C; for *tetO* (515 bp) detection, the annealing temperature was changed to 55°C. For *tetK* (172 bp) and *tetL* (993 bp), the amplification protocol consisted of 35 cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C. For *ermA* (645 bp), *ermB* (639 bp), *ermC* (642 bp), *ermM* (658 bp), and *ermTR* (385 bp), the 35 amplification cycles consisted of 1 min at 94°C, 1 min at 52°C, 55°C, 46°C, 50°C, and 48°C, respectively, and 1 min at 72°C. For *mefA*, the amplification step consisted of 35 cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C.

AG-modifying enzyme genes. The presence of *aac(6′)-aph(2′)*, *aph(3′)-IIIa*, and *ant(4′)-Ia* genes was detected by PCR by a standard protocol. Each reaction was performed in a final volume of 50 μl containing 0.4 μM concentrations of each primer, 200 μM concentrations of each deoxynucleoside triphosphate, 100 ng of DNA, and 1 U of *Taq* polymerase in 1× buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 1.5 mM $MgCl_2$). The specific primers used for the present study are shown in Table 1.

Types of disease. (i) Early onset disease was defined as disease that occurs in the setting of maternal complications in the first week of life. (ii) Late-onset disease was defined as disease that affects term infants, with an unremarkable maternal and early neonatal course (≥7 days to 90 days). (iii) "Late, late-onset" disease is late-onset infection with a delayed onset (>90 days). (iv) Infections localized in deep tissues, blood, cerebrospinal fluid (CSF), or other liquids obtained by punction, whose microbiological diagnosis was performed by using organisms isolated from otherwise-sterile samples, were defined as invasive infections.

RESULTS

Origin and source of isolates. Fifty-eight cases, originating from the following cities, were studied: Buenos Aires City and the surrounding area, 29 (11 nonevaluable cases); Rosario, 11 (two nonevaluable cases); Bahía Blanca, 5; Mar del Plata, 4 (one nonevaluable case); La Plata, 3 (two nonevaluable cases); Goya, 3 cases; Neuquén, 2; and Salta, 1. No cases were recorded in Tres Arroyos, Tandil, Esquel, and Córdoba despite the screening for all potential cases occurring in the three first cities. Epidemiological data were available for 44 patients (31 adults and 13 children).

Twenty-six GBS were isolated from blood cultures, followed by skin and soft tissues, bones and joint, and CSF. In four instances, GBS strains were isolated simultaneously from two kinds of samples each: blood and CSF ($n = 3$) and blood and joint ($n = 1$) (Table 2).

Pediatric patients. Twelve cases were recorded in newborns (six males and six females), and one fatal case was detected in a 5-month-old boy, considered a case of "late, late-onset neonatal disease." Early-onset disease was present in eight patients: five with respiratory distress, one with respiratory distress and meningitis, and two with primary bacteremia.

Late-onset disease was observed in four neonates: three with meningitis and one with osteomyelitis.

Adult patients. The mean age of the adult patients was 58 years, with 23 (74.2%) males and 8 (35.8%) females. In adult patients, GBS strains were frequently involved in skin, soft tissue, and bone and joint infections (61.3% as a whole), and

TABLE 1. Primers used in this study

Gene	Primer		Product size (bp)	GenBank access no. and/or reference
	Name	Sequence		
tetM	tetmF	5′-TTATCAACGGTTTATCAGG-3′		
	tetmR	5′-CGTATATATGCAAGACG-3′	397	1
tetO	tetoF	5′-AACTTAGGCATTCTGGCTCAC-3′		
	tetoR	5′-TCCCACTGTTCCATATCGTCA-3′	515	2
tetK	tetkF	5′-TCCTGGAACCATGAGTGT-3′		
	tetkR	5′-AGATAATCCGCCCATAAC-3′	189	S74032; this study
tetL	tetlF	5′-TGAACGTCTCATTACCTG-3′		
	tetlR	5′-ACGAAAGCCCACCTAAAA-3′	993	U17153; this study
ermA	ermaF	5′-TCTAAAAAGCATGTAAAAGAA-3′		
	ermaR	5′-CTTCGATAGTTTATTAATATTAGT-3′	645	3
ermB	ermBF	5′-GAAAAGGTACTCAACCAAATA-3′		
	ermBR	5′-AGTAACGGTACTTAAATTGTTTAC-3′	639	3
ermC	ermCF	5′-TCAAAACATAATATAGATAAA-3′		
	ermCR	5′-GCTAATATTGTTTAAATCGTCAAT-3′	642	3
ermM	ermMF	5′-TCGGCTCAGGAAAAGGG-3′		
	ermMR	5′-CAAGTTAAGGATGCAGT-3′	658	M12730; this study
ermTR	ermTRF	5′-TTGGGTCAGGAAAAGGA-3′		
	ermTRR	5′-GGGTGAAAATATGCTCG-3′	385	AF002716; this study
mefA	mefAF	5′-CGTAGCATTGGAACAGC-3′		
	mefAR	5′-TGCCGTAGTACAGCCAT-3′	316	U70055; this study
mefE	mefEF	5′-CGTAGCATTGGAACAGC-3′		
	mefER	5′-TCGAAGCCCCCTAATCTT-3′	513	U83667; this study
msrA	msrAF	5′-CACGTTAGACGGTAGTTT-3′		
	msrAR	5′-TTCGTTCTTTCCCCACC-3′	1,000	AB016613; this study

only 19.4% of cases were primary bacteremia. The most frequent underlying diseases were diabetes ($n = 7$, 22.6%) and tumors ($n = 4$, 12.9%).

Mortality. Mortality could be only assessed in 37 patients (26 adults and 11 children) because in seven cases the outcome remained unknown. Overall mortality was 13.5%, including one 5-month-old boy (9% in children) and four adult patients (15.4% in adults). One of them was a 30-year-old man with AIDS, pneumonia, and ascitis, who died suddenly as a consequence of a bacteremia due to GBS. The other three adults were elderly patients (an 83-year-old man, a 70-year-old man, and an 82-year-old woman) with underlying diseases. One of those deaths, an 83-year-old patient with a prostatic carcinoma, was clearly not associated with the presence of GBS in

TABLE 2. Distribution of serotypes of 44 GBS isolates obtained from invasive infections in Argentina according to source

Source(s)	No. of serotypes[a]									
	Ia	Ia/c	Ib/c	II	II/c	III	IV	IV(w)	NT	Total
Skin and soft tissue				3		2	1	1		7
Bone and joint			1	1					3	5
Blood	3	6		6	1	3		3		22
Blood and joint						1				1
Blood and CSF		1				2				3
CSF						2				2
Pleural fluid						1				1
Other[b]	1	2								3
Total	4	9	1	10	1	11	1	4	3	44

[a] In five cases, GBS isolates were obtained from more than one site. NT, nontypeable. Serotypes V, VI, VII, and VIII were not included in this table. Serotype V was not found among evaluable patients, while serotypes VI, VII, and VIII were not found in the whole study. Isolates reacting weakly with their respective antisera were included as IV(w).

[b] That is, abdominal fluid ($n = 1$) and kidney punction ($n = 2$).

TABLE 3. Distribution of serotypes of 44 GBS isolates obtained from invasive infections in Argentina according to patient age

Patient group	No. of serotypes[a]									
	Ia	Ia/c	Ib/c	II	II/c	III	IV	IV(w)	NT	Total
Newborns (early)	1	3		1		3				8
Newborns (late)						4				4
Children					1					1
Adults[b]	2	2	1	7		3	1	3	3	22
Elderly adults[c]	1	4		1		2		1		9
All adults	3	6	1	8		5	1	4	3	31

[a] See Table 2, footnote *a*.
[b] That is, adults 18 to 69 years old.
[c] That is, adults ≥70 years old.

the sample of soft tissue, whereas the other two cases may be considered related to streptococcal bacteremia. One was a 70-year-old man who developed septic shock with hepatic and kidney failure after a hip replacement. The other case was an 82-year-oold woman with cardiovascular failure and erisipela. In consequence, the mortality attributable to invasive infections of GBS was 11.5% in adults and 9% in children (10.8% as a whole).

Serotype analysis. All 58 GBS isolates of equal numbers of patients were available for serotyping. Overall, 5 of the isolates were serotype Ia, 14 were serotype Ia/c, 1 was serotype Ib/c, 10 were serotype II, 2 were serotype II/c, 13 were serotype III, 5 were serotype IV, 2 were serotype V, and 6 were not typeable. Serotypes VI, VII, and VIII were not found in the present study. The serotype distribution in GBS isolated from the evaluable patients is presented in Table 3. Twelve strains were obtained from newborns. Eight GBS strains of four different serotypes (one serotype Ia, three serotype Ia/c, one serotype II, and three serotype III) were obtained from early-onset infections (61.5%), and four group III GBS strains were obtained from late-onset infections (30.8%) (Table 3). One serotype II/c (7.7%) strain was isolated from a fatal case (a 5-month-old boy).

No prevalence of certain serotypes was observed except for that of serotype III in CSF (Table 2). Serotype V was only found in isolates from blood cultures of two nonevaluable patients.

Thirty-one isolates were obtained from adults with a mean age of 58.8 years (range, 21 to 83 years). In spite of the low number of isolates in each group, the serotypes appeared to be evenly distributed in <70-year-old adults ($P > 0.05$), with the exception of serotype II, the most frequently found ($n = 7$). Serotypes IV and I ($n = 4$ each), serotype III and nontypeable isolates ($n = 3$ each), and serotype Ib/c ($n = 1$) were also present (Table 3). Fatal cases in adults were associated with serotypes II ($n = 1$), Ia ($n = 1$), and Ia/c ($n = 2$).

Antimicrobial susceptibility. All GBS isolates were susceptible to PEN and CRO (with a 90% MIC [MIC_{90}] for both antibiotics of ≤0.007 μg/ml). Resistance to CLI ($n = 1$, 1.7%), ERY ($n = 3$, 5.2%), AZM ($n = 3$, 5.2%), MIN ($n = 40$, 69.0%), and TET ($n = 42$, 72.4%) was detected. Two ERY-resistant isolates of serotypes Ia and IV presented the M phenotype; the presence of the *mefA* gene, the determinant of an active efflux mechanism, was found in both isolates. The other ERY-resistant isolate (serotype V) has the *ermTR* gene. It was also resistant to CLI, showing the constitutive MLS phenotype. TET-resistant isolates showed MICs between 16 and 64 μg/ml. All of them were simultaneously resistant to MIN (MIC = 8 to 64 μg/ml), except for two MIN-susceptible strains (MIC = 2 μg/ml) and one intermediately MIN-susceptible isolate (MIC = 4 μg/ml). Genetic determinants for TET-resistant were identified in 41 studied isolates: 36 showed the *tetM* gene, 3 showed the *tetO* gene, and 2 showed the *tetM* and *tetO* genes simultaneously.

Only one isolate, SR362 (serotype III), was recognized as resistant to at least one AG. It was obtained from the foot of a diabetic 57-year-old man as part of the polymicrobial flora. He was successfully treated with ciprofloxacin plus metronidazole during 15 days and clindamycin plus cephalexin during 38 days.

In the disk diffusion test, we observed a diminished zone with 120-μg GEN disks related to other isolates and no zone with 120-μg KAN disks, as with other GBS isolates (Table 4). Using Etest with standard KAN and AMK strips, no differences between strains were found, but a subtle increase was noted in the MIC of SR362 (128 μg/ml versus 32 μg/ml with other isolates) when we used high-level GEN Etest strips.

Differences in MICs of KAN and AMK only could be observed when the agar dilution method was used. Looking for an easier method, we used commercial 1,000-μg KAN disks, an approach currently used to help in the identification of anaerobes, and we obtained significant differences between SR362 and other GBS strains tested (Table 4).

Strain SR362 behaved similarly to other isolates in response to highly loaded STR disks and STR Etest strips (MIC = 256 μg/ml for all of the isolates) (Table 4).

Time-kill curves were determined in all 44 GBS isolates showing synergy between PEN and all tested AGs with 43 isolates. Only synergy was impaired for SR362 when we used GEN, KAN, and AMK, but not when we used STR. SR362 was characterized by a PCR method as having the gene *aac6/aph2*.

DISCUSSION

Almost 30 years ago, GBS emerged as the leading cause of perinatal infectious diseases (43). Approximately 60 to 80% of infant infections were early-onset diseases in different studies in the United States (3, 43). In the present study, 46.1% of these infections were early-onset infections.

All adult patients included in the study were males or nonpregnant women. Invasive GBS disease occurring outside the perinatal period was recognized as a growing problem espe-

TABLE 4. AG susceptibility of strain SR362 compared to the susceptibility of 43 other GBS isolates obtained from invasive infections

Antibiotic	Disk diffusion results			MIC (μg/ml) as determined by:				Synergy with PEN[a]	
	Disk content (μg)	Zone diam (mm)		Etest		Agar dilution			
		SR362	Other[b]	SR362	Other[b]	SR362	Other[b]	SR362	Other[b]
GEN	120	10	15–20	128	32	128	32	No	Yes
KAN	120	6	6–15	>256	>256	>2,048	256–512	No	Yes
	1,000	6	17–20						
AMK	NT[c]	NT	NT	>256	128–>256	>2,048	256	No	Yes
STR	300	15	14–22	256	256	256	256	Yes	Yes

[a] Synergy was considered when the bacterial concentration diminished >2 logs for the combination respective to PEN after 6 h of incubation at 35°C.
[b] That is, the other 57 isolates obtained during the study.
[c] NT, not tested.

cially for debilitated hosts. In the metropolitan area of Atlanta, Ga., it was found that diabetes mellitus and cancer were risk factors for community acquired GBS infections (11). In the present study, malignancies (12.9%) and diabetes (22.6%) were the two most frequently found underlying diseases. The mean age of adult patients was 58 years, which is similar to what was found in two studies conducted in Atlanta: 59 years (11) and 62 years (45).

GBS was frequently isolated in skin, soft tissues, and bone samples from adult patients as in previous reports (3, 11). Also, bacteremia with no identified source was frequently found in such patients (19.4% here versus 30% in the earlier study) (11).

Mortality was not observed in neonates. However, fatal infections occurred in one 5-month-old infant, in a young man with AIDS, and in three elderly adult patients with underlying predisposing conditions (15.4% in adults and 9% in children). Mortality attributable to GBS was 11.5% in adults and 9% in children. In comparison, mortality was estimated to be 4 to 15% in early-onset, 0 to 6% in late-onset, and <5% in late, late-onset disease in neonatal sepsis; 8 to 70% in earlier studies of adult patients; and 20 to 30% in recent studies (3). Among adults in Atlanta, mortality was 32% in 1982 and 1983, whereas in the other more recent study it was 21% (11, 45).

As previously observed by others, serotypes Ia (including Ia/c) and III were the most prevalent serotypes globally (3). However, serotype II, uncommonly found in human infections, was frequently isolated in the present study. Serotypes IV and NT strains were only found in adults, whereas serotypes VI, VII, and VIII, commonly found in Japan (15), were not observed among the 58 GBS isolates isolated in the present Argentinian study. As previously mentioned, serotype III predominated in late-onset neonatal disease, and serotypes Ia and III were the most commonly identified serotypes among patients with early-onset disease, although serotype II was also seen in our study (30). A moderately high prevalence of serotype II, both in early-onset diseases in newborns and in nonpregnant adult infections, was also noted in Belgium (26). Serotype V, one of the most prevalent serotypes in the United States, was only found in two nonevaluable cases.

The most frequent clinical presentations of early-onset disease were septicemia without focus ($n = 2$), pneumonia ($n = 5$), and pneumonia plus meningitis ($n = 1$). The most frequent clinical presentations of late-onset disease were meningitis ($n = 3$) and osteomyelitis ($n = 1$). Meningitis always was due to serotype III GBS, as expected (43).

One fatal case of sepsis in a 5-month-old boy can be interpreted as a late, late-onset neonatal sepsis according to Baker (3). Its immunologic status could not be studied because the boy died abruptly a few hours after hospitalization. Possible foci were a leg ulcer and an enteral focus (diarrhea).

In adults less than 70 years old, serotype II was the most commonly found, whereas serotype Ia (including Ia/c) and, less frequently, serotype III were the most prevalent in elderly adults (Table 3).

Overall, in our experience serotype I, including serotype Ia/c (9 of 31), and serotype II (8 of 31), were the two most frequently found GBS serotypes in adults, followed by serotype III. Serotypes Ia, III, and V were described elsewhere as the most common serotypes in adult GBS diseases (3, 6). Our data differed from that of the literature since serotype V was only found in two nonevaluable patients.

As expected, in the present study neither PEN-resistant nor intermediately PEN-susceptible GBS strains were found (MIC_{90} = 0.007 μg/ml). Likewise, all isolates were also susceptible to CRO. As in previous reports, high percentages of TET-resistant GBS were detected. Our results (72.4%) were similar to those recorded in several studies performed in different countries: Anthony et al. (United States) (1), 85.3%; Traub and Leonhard (Germany) (48), 74.5%; De Azavedo et al. (Canada) (9), 82 to 87%; De Mouy et al. (France) (10), 88.1%; Betriú et al. (Spain) (4), 89%; Melin et al. (Belgium) (25), 74.2 to 86.7%; and Fujita et al. (Japan) (14), 71.9%. However, another Japanese study that included new serotypes found only 26% of strains were resistant to TET (24). Thirty-two of the TET-resistant isolates harbored only the *tetM* gene. All of them were also resistant to MIN. Three TET-resistant isolates harbored only the *tetO* gene: one was susceptible, one was intermediately susceptible, and one was resistant to MIN. Three TET-resistant and MIN-resistant isolates harbored both the *tetO* and the *tetM* gene. One of the isolates that was TET resistant but MIN susceptible did not show any of the tested mechanisms.

ERY resistance was detected in 5.2% of the invasive GBS isolates studied. This rate can be compared to Japanese (3.1 to 3.0%) (14, 24) and German (4.9 and 12%) (41, 48) data, but it

was higher than rates reported in Morocco (27) and lower than rates reported in Spain (8.0, 14.7, and 18.0%) (4, 38; C. Betriú, E. Culebras, I. Rodríguez-Avial, M. Gómez, B. A. Sánchez, and J. J. Picazo, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. C2-234, 2001), the United States (7.4 to 16%) (12, 28, 34), France (18.0 to 21.4%) (10, 13), and Canada (18.0%) (9). M and MLS resistance phenotypes were observed in two and one ERY-resistant isolates of different serotypes, respectively. Other authors have highlighted the prevalence of MLS resistance phenotype over M phenotype in GBS (10, 27, 34, 38).

In *Streptococcus* spp. MLS resistance is usually mediated by *ermAM*/*ermB* class genes (18, 19). The *ermTR* gene was described for the first time in Finland by Kataja et al. in group A streptococci (17, 19). The nucleotide sequence of *ermTR* is 83% identical to that of *ermA* described in *Staphylococcus aureus* and in coagulase-negative staphylococci, and it was recently included among the *ermA* denomination (39). In our bacterial population, the isolate that has an MLS resistance phenotype harbored the *ermTR* gene.

The MICs of AZM were slightly higher than those of ERY, but the MICs were roughly equivalent (30, 31).

One anomalous highly AG-resistant strain was described in the present study. To our knowledge, only a few AG-resistant GBS have been reported, making SR362 the second GBS isolate in which the bifunctional 6′-acetyltransferase-2″-phosphotransferase enzyme was found (7, 20). This strain behaved as a GEN-susceptible strain, considering the breakpoints established for enterococci (MIC = 128 μg/ml). However, it cannot be synergically killed by the combination PEN-GEN. Moreover, according to the specificity of the enzyme, PEN-KAN and PEN-AMK were combinations equally inactive. The MICs of KAN and AMK could predict their failure but only by the agar dilution method or by the use of the hypercharged KAN disks (1,000 μg) currently used to identify anaerobic bacteria. Highly charged KAN or AMK Etest strips are not currently available, and regular strips, with scales from 0.016 to 256 μg/ml, were not able to distinguish between susceptible isolates for which the antibiotic MICs are 256 to 512 μg/ml and resistant isolates for which the antibiotic MICs are >2,048 μg/ml.

We think that strains such as SR362 may be overlooked if conventional methods are applied to analyze GBS isolates. We propose, as a possible screening method for strains with the bifunctional enzyme, the use of 1,000-μg KAN disks or 120-μg GEN disks but, in the latter case, we propose changing the current breakpoints used for enterococci. Recently, a urinary isolate was recognized as highly resistant to GEN and KAN as determined by using only 1,000-μg KAN disks (unpublished observations). For 1,000-μg KAN disks, no zone should be indicative of resistance, and zones of ≥17 mm should be considered susceptible. Using GEN disks, zones of ≥15 or ≤10 mm should be considered susceptible or resistant, respectively.

ACKNOWLEDGMENTS

The Argentinian Streptococcus Study Group is comprised of 40 Argentinian centers: 19 from Buenos Aires City (C. Hernández, Hospital de Pediatría J. P. Garrahan; J. Kovensky, Hospital de Quemados; A. Meo, Hospital Ramos Mejía; M. J. Rial and G. Ojea, Hospital Pedro Elizalde; M. López, Hospital Santojanni; L. López Moral and M. Badía, Hospital Cosme Argerich; H. Villar and M. Hoffman, Hospital Tornú; M. Quinteros and E. Couto, Hospital F. J. Muñiz; A. M. Casimiro, Hospital Pirovano; O. Sívori, IREP; M. Ferreiro and M. Flaibani, Hospital Durand; S. Kaufman, Hospital J. Fernández; M. Iglesias and C. Schijman, Hospital Alvarez; L.Targa, Hospital Aeronáutico; M. Tokumoto, Fundación Favaloro; C. Ebi, Sanatorio Anchorena; A. Bau and G. Rodríguez, Sanatorio Santa Isabel; and A. Sucari, Clínica del Sol), 3 from La Plata (A. Aguirre, IPENSA; C. Lopreto, J. Viegas Caetano, Hospital San Martín; and G. Ramírez Gronda, Hospital Sor María Ludovica), 2 from Bahía Blanca (S. Calcagni, Laboratorio Integrado, and M. Rizzo and S. Vaylet, Hospital Penna), 4 from Rosario (E. Sutich, IPAM; J. P. Scapini and O. Teglia, Sanatorio Parque; D. Ingaramo and R. Ingaramo, Hospital Italiano; and J. P. Scapini and L. Flynn, Sanatorio de Niños), 2 from Neuquén (S. Brasili, Hospital B. Roldán, and V. Soriano, Policlínico Neuquén), and 1 each from Mar del Plata (S. Bulacio, M. Vallejo, and L. De Wouters, Hospital Privado de Comunidad), Goya (D. Benedí and E. González, Laboratorio de la Municipalidad), Salta (J. Mulki and M. S. Rabich, Hospital Materno-Infantil), Tres Arroyos (N. G. Pagniez and J. Calabrese, Hospital Pirovano), Lanús (M. Giangreco and B. Mizraji, Hospital Evita), Los Polvorines (P. Vidal, Hospital Ramón Carrillo), Tandil (M. Sparo Hospital Ramón Santamarina), Guernica (S. Mattoni, Hospital Cecilia Grierson), Ezeiza (R. Pereyra, Hospital de Ezeiza), Esquel (O. Daher, Hospital de Esquel), and Córdoba (S. Moreno and A. Littvik, Hospital Rawson).

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