ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 2005, p. 3080–3082 Vol. 49, No. 7
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.7.3080–3082.2005

Heterogeneity of Genotype-Phenotype Correlation among Macrolide-Resistant *Streptococcus agalactiae* Isolates

Ulrich von Both, Anna Buerckstuemmer, Kirsten Fluegge, and Reinhard Berner*

Department of Pediatrics and Adolescent Medicine, University Hospital of Freiburg, Mathildenstrasse 1, D-79106 Freiburg, Germany

Received 10 February 2005/Returned for modification 27 March 2005/Accepted 20 April 2005

Seventy-four erythromycin-resistant group B *Streptococcus* isolates were analyzed regarding their phenotype-genotype and phenotype-serotype correlation. Four different phenotypes were assessed, one of them for the first time. *ermB* and *ermTR* were the most frequent genotypes (80%). The most prevalent serotype III showed great phenotypic variability while serotype V was strongly associated only with two different phenotypes.

Streptococcus agalactiae (group B *Streptococcus* [GBS]) is a well-known cause of invasive infections in neonates (3, 15). For treatment or prevention of GBS disease, erythromycin and clindamycin are recommended second-line drugs for patients with β-lactam allergy (1, 5). In recent years, increasing macrolide resistance rates have been reported for several streptococcal populations worldwide, which is of significant clinical relevance since macrolides are among the most frequently used antibiotics, especially in the pediatric population (9, 11, 12, 13, 14). This resistance is genetically based on either the modification of the ribosome encoded by *erm* genes (macrolide-lincosamide-streptogramin B [MLS_B] phenotype with inducible [i] or constitutive [c] expression) or the efflux of macrolide antibiotics by a *mefA*-encoded efflux pump (M phenotype). The correlation of specific antimicrobial susceptibility patterns and the respective macrolide resistance genes in *s*treptococci has been an important subject for investigation worldwide (18, 19). To analyze the phenotype-genotype as well as the phenotype-serotype correlation among macrolide resistant GBS isolates, the present study focused on a collection of 74 erythromycin-resistant GBS strains from a southwestern region of Germany collected between the years 1999 and 2004 with 27 of them described previously (20). Briefly, 74 erythromycin-resistant GBS isolates of neonatal origin (cultured from blood, cerebrospinal fluid, urine, and swab cultures) as well as from vaginal swabs of pregnant women were identified using β-hemolysis testing as well as the Pastorex Strep B agglutination test (Bio-Rad, Marnes-la-Coquette, France). Isolates were subsequently phenotypically analyzed by means of the disk diffusion method and screened by PCR for erythromycin resistance genes (*mefA* or *mefE*, *ermB*, and *ermTR*) as well as *linB* using primers previously described (4, 6, 16, 17). PCR assays were reproducibly repeated at least three times for every strain. To differentiate between specific MLS phenotypes, a triple-disk test using erythromycin, clindamycin, and josamycin was performed as previously described (10). Serotyping was performed using an enzymatic extraction method (2).

Results of triple-disk testing revealing four distinct phenotypes are shown in Fig. 1. Thirty-nine isolates (52%) showed the cMLS phenotype (P1) mostly correlated with the *ermB* resistance gene. Twenty isolates (27%) showed an inducible resistance phenotype (iMLS) with 13 strains exhibiting inducible resistance to clindamycin and josamycin associated with the *ermTR* gene (P3) and another 7 strains displaying constitutive resistance to clindamycin as well as inducible resistance to josamycin based on either the *ermB* or the *ermTR* gene (P2). The M phenotype (P4) could be detected in 15 isolates (20%) strongly associated with the *mefA* gene (Table 1). All three phenotypes (P1, P3, and P4) correspond well to the ones previously described by different authors (10, 19) while P2, in contrast, has not been described so far. The most prevalent serotypes (ST) among all erythromycin-resistant GBS isolates were ST V (37%) and III (27%) (Table 2). While ST III showed a great variety of different phenotypes, including P2 and P4 (M phenotype), ST V revealed only two different MLS phenotypes (P1 and P3) strongly associated with the *ermB* or *ermTR* gene, respectively. Since different studies described the clonal spread of ST V isolates within the GBS population in recent years (8, 20), this might be an explanation for the observed homogeneity of this respective ST V population compared to the ST III isolates in this study. Regarding the newly described P2 phenotype, serotypes Ib and II as well as the predominant serotype III (four of seven isolates) were detected. PCR analysis of these seven isolates revealed either the *ermB* or the *ermTR* gene while PCR on *linB*, a clindamycin resistance gene previously described in GBS by de Azavedo et al. (7) yielded negative results. To our knowledge, there are no data in present literature describing this P2 phenotype as well

TABLE 1. Distribution of resistance genes among four different phenotypes, P1 to P4[a]

Phenotype	No. of isolates with gene(s):					
	ermB	*ermTR*	*mefA*	*ermB* + *ermTR*	*ermTR* + *mefA*	Total
P1	**34**	3		**2**		39
P2	3	4				7
P3	1	**9**		**2**	1	13
P4			**15**			15
Total	38	17	15	4	1	74

[a] Boldface indicates the predominant genotype in the respective phenotype.

* Corresponding author. Mailing address: Department of Pediatrics and Adolescent Medicine, University Hospital of Freiburg, Mathildenstrasse 1, D-79106 Freiburg, Germany. Phone: 49-761-270-4480. Fax: 49-761-270-4598. E-mail: berner@kikli.ukl.uni-freiburg.de.

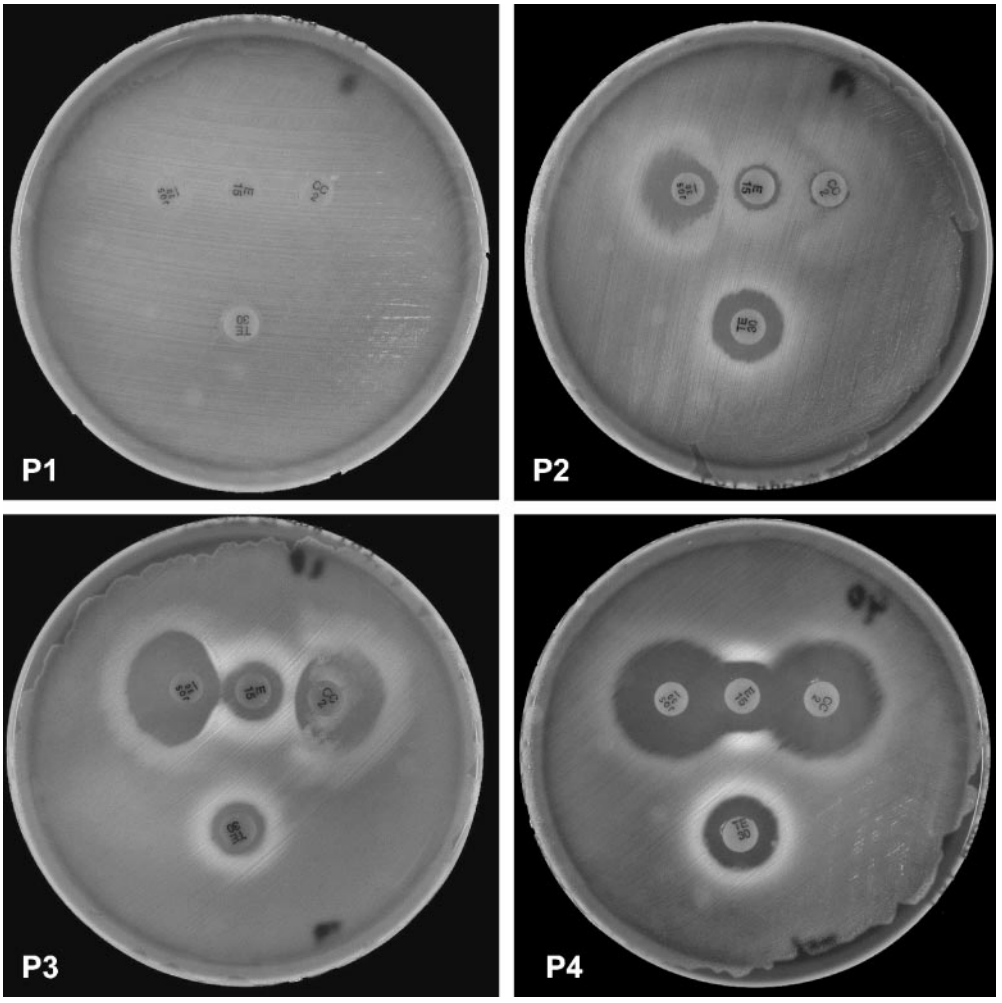

FIG. 1. Phenotypes of erythromycin-resistant *S. agalactiae* isolates determined by the triple-disk test. The erythromycin disk (30 μg) is at the center, with the clindamycin disk (10 μg) on the right and the josamycin disk (30 μg) on the left. P1, cMLS phenotype; P2 and P3, iMLS phenotypes; P4, M phenotype.

as its genetic determinant. It is likely to presume that other to date unknown resistance factors or a certain combination of those with known resistance genes is responsible for the heterogeneity of phenotype-genotype correlation among GBS isolates. Such a variety of resistance factors possibly acquired through horizontal gene transfer might as well be responsible for the reported rapid increase of macrolide resistance among GBS isolates.

TABLE 2. Distribution of serotypes among four different phenotypes, P1 to P4[a]

Phenotype	No. of isolates with serotype:							
	Ia	Ib	II	III	IV	V	NT	Total
P1	3	2	1	7	2	**22**	2	39
P2		1	1	4			1	7
P3	1	1		3	2	**6**		13
P4	4	2	2	6	1			15
Total	8	6	5	**20**	5	**28**	3	74

[a] Boldface indicates the predominant serotype in the respective phenotype. NT, nontypeable.

REFERENCES

1. **American Academy of Pediatrics Committee on Infectious Disease and Committee on Fetus and Newborn.** 1997. Revised guidelines for prevention of early-onset group B streptococcal (GBS) infection. Pediatrics **99:**489–496.
2. **Berner, R., A. Bender, C. Rensing, J. Forster, and M. Brandis.** 1999. Low prevalence of the immunoglobulin-A-binding β antigen of the C protein among *Streptococcus agalactiae* isolates causing neonatal sepsis. Eur. J. Clin. Microbiol. Infect. Dis. **18:**545–550.
3. **Berner, R., R. F. Schumacher, S. Bartelt, J. Forster, and M. Brandis.** 1998. Bacteremia in hospitalized children: predisposing conditions and case-related microorganisms. Eur. J. Clin. Microbiol. Infect. Dis. **17:**337–340.
4. **Bozdogan, B., L. Berrezouga, M.-S. Kuo, D. A. Yurek, K. A. Farley, B. J. Stockman, and R. Leclercq.** 1999. A new resistance gene, *linB*, conferring resistance to lincosamides by nucleotidylation in *Enterococcus faecium* HM1025. Antimicrob. Agents Chemother. **43:**925–929.
5. **Centers for Disease Control and Prevention.** 1996. Prevention of perinatal group B streptococcal disease: a public health perspective. Morbid. Mortal. Wkly. Rep. **45:**1–24.
6. **Clancy, J., J. Petitpas, F. Dib-Hajj, W. Yuan, M. Cronan, A. V. Kamath, J. Bergeron, and J. A. Retsema.** 1998. Molecular cloning and functional analysis of a novel macrolide-resistance determinant, mefA, from *Streptococcus pyogenes*. Mol. Microbiol. **42:**1493–1494.
7. **de Azavedo, J. C. S., M. McGavin, C. Duncan, D. E. Low, and A. McGeer.** 2001. Prevalence and mechanisms of macrolide resistance in invasive and noninvasive group B streptococcus isolates from Ontario, Canada. Antimicrob. Agents Chemother. **45:**3504–3508.
8. **Elliot, J. A., K. D. Farmer, and R. R. Facklam.** 1998. Sudden increase in

isolation of group B streptococci, serotype V, is not due to emergence of a new pulsed-field gel electrophoresis type. J. Clin. Microbiol. **36:**2115–2116.

9. **Fernandez, M., M. E. Hickman, and C. J. Baker.** 1998. Antimicrobial susceptibility of group B streptococci isolated between 1992 and 1996 from patients with bacteremia and meningitis. Antimicrob. Agents Chemother. **42:**1517–1519.
10. **Giovanetti, E., M. P. Montanari, M. Mingoia, and P. E. Varaldo.** 1999. Phenotypes and genotypes of erythromycin-resistant *Streptococcus pyogenes* strains in Italy and heterogeneity of inducibly resistant strains. Antimicrob. Agents Chemother. **43:**1935–1940.
11. **Lin, F. Y. C., P. H. Azimi, L. E. Weisman, J. B. Philips III, J. Regan, P. Clark, C. G. Rhoads, J. Clemens, J. Troendle, E. Pratt, R. A. Brenner, and V. Gill.** 2000. Antibiotic susceptibility profiles for group B streptococci isolated from neonates, 1995–1998. Clin. Infect. Dis. **31:**76–79.
12. **Morales, W. J., S. S. Dickey, P. Bornick, and D. V. Lim.** 1999. Change in antibiotic resistance of group B streptococcus: impact on intrapartum management. Am. J. Obstet. Gynecol. **181:**310–314.
13. **Pearlman, M. D., C. L. Piersoni, and R. G. Faix.** 1998. Frequent resistance of clinical group B streptococci isolates to clindamycin and erythromycin. Obstet. Gynecol. **92:**258–261.
14. **Rouse, D. J., W. W. Andrews, F. Y. C. Lin, C. W. Mott, J. C. Ware, and J. B. Philips III.** 1998. Antibiotic susceptibility profile of group B streptococcus acquired vertically. Obstet. Gynecol. **92:**931–934.
15. **Schrag, S. J., S. Zywicki, M. M. Farley, A. L. Reingold, L. H. Harrison, L. B. Lefkowitz, J. L. Hadler, R. Danila, P. R. Cieslak, and A. Schuchat.** 2000. Group B streptococcal disease in the era of intrapartum prophylaxis. N. Engl. J. Med. **342:**15–20.
16. **Seppälä, H., M. Skurnik, H. Soini, M. C. Roberts, and P. Huovinen.** 1998. A novel erythromycin resistance methylase gene (*ermTR*) in *Streptococcus pyogenes*. Antimicrob. Agents Chemother. **42:**257–262.
17. **Sutcliffe, J., T. Grebe, T. Tait-Kamradt, and L. Wondrack.** 1996. Detection of erythromycin-resistant determinants by PCR. Antimicrob. Agents Chemother. **40:**2562–2566.
18. **Uh, Y., I. H. Jang, G. Y. Hwang, M. K. Lee, K. Y. Yoon, and H. Y. Kim.** 2004. Serotypes and genotypes of erythromycin-resistant group B streptococci in Korea. J. Clin. Microbiol. **42:**3306–3308.
19. **Uh, Y., I. H. Jang, G. Y. Hwang, M. K. Lee, K. J. Yoon, and H. Y. Kim.** 2004. Antimicrobial susceptibility patterns and macrolide resistance genes of β-hemolytic streptococci in Korea. Antimicrob. Agents Chemother. **48:**2716–2718.
20. **von Both, U., M. Ruess, U. Mueller, K. Fluegge, A. Sander, and R. Berner.** 2003. A serotype V clone is predominant among erythromycin-resistant *Streptococcus agalactiae* isolates in a southwestern region of Germany. J. Clin. Microbiol. **41:**2166–2169.