Susceptibility to antimicrobials and mechanisms of erythromycin resistance in clinical isolates of *Streptococcus agalactiae* from Rio de Janeiro, Brazil

Streptococcus agalactiae (group B *Streptococcus*; GBS) is widely recognized as one of the major aetiological pathogens in neonatal sepsis and meningitis. It is also an important cause of pregnancy-related infections and invasive infections in non-pregnant adults (Muñoz *et al.*, 1997).

Penicillin is recommended as the treatment of choice and is prophylactic for the prevention of group B streptococcal infections in neonates (Centers for Disease Control and Prevention, 1996). Macrolides are a useful alternative therapy in penicillin-allergic patients; however, emergence of strains resistant to macrolides has been observed in several countries, at a frequency ranging from 2 to 46% (Traub & Leonhard, 1997; Hsueh *et al.*, 2001).

There have been no studies to investigate macrolide-resistance mechanisms in GBS from Brazil. Therefore, the aim of this study was to determine susceptibility to antimicrobial agents among 92 *S. agalactiae* isolates that were recovered from various clinical specimens (mainly urine, blood, cerebrospinal fluid and endocervix) collected between 1994 and 1999 in Rio de Janeiro, Brazil, in order to detect emerging resistance, to determine phenotypic and genotypic mechanisms of macrolide resistance and to study genetic relatedness among macrolide-resistant GBS strains. Isolates were identified by conventional methods and tested for susceptibility to antimicrobial agents by the agar disc-diffusion technique (National Committee for Clinical Laboratory Standards, 2000a). GBS isolates were consistently susceptible to cefepime, chloramphenicol, penicillin and vancomycin.

Trimethoprim/sulfamethoxazole and tetracycline resistance was common among GBS isolates (36 and 87%, respectively). Erythromycin resistance was detected in four (4.3%) of 92 isolates by the disc-diffusion assay. By agar-dilution assay for MIC determination (National Committee for Clinical Laboratory Standards, 2000b),

we observed that the results were similar to those of the disc-diffusion assay, except for one isolate that showed intermediate susceptibility to erythromycin by the disc-diffusion test and resistance by MIC determination. Similar discrepancy has been reported before (Traub & Leonhard, 1997). Total erythromycin resistance in the period of study was 5.4%, which is similar to values reported recently in other countries (Fernandez *et al.*, 1998; de Azavedo *et al.*, 2001). For clindamycin, only one isolate (1.1%) was resistant; this isolate showed high-level resistance. Among the erythromycin-resistant isolates that were subjected to the double-disc test, carried out as described by Seppälä *et al.* (1993), three isolates expressed the iMLS_B phenotype, one isolate expressed the cMLS_B phenotype and one isolate showed the M phenotype. The latter strain was recovered in 1997 and was the only strain that showed intermediate susceptibility by the disc-diffusion test.

As detected by a PCR assay that was described previously (d'Oliveira *et al.*, 2003), erythromycin resistance in GBS isolates was associated mainly with the *erm* genes, predominantly the *ermTR* gene (*ermA*, amplified with the primer described by Seppälä *et al.*, 1998), but the *ermB* and *mefA/E* genes [both amplified with primers suggested by Sutcliffe *et al.* (1996)] were also found in the cMLS_B and M phenotype isolates, respectively, showing total correlation between erythromycin-resistance phenotypes and genetic mechanisms. None of the isolates carried both the *erm* and *mef* genes. Clonal relatedness, based on *Sma*I-digested DNA profiles by PFGE [determined according to d'Oliveira *et al.* (2003)], showed a high degree of genetic heterogeneity, suggesting the importance of epidemiological surveillance of GBS infections and of continuous monitoring of the resistance characters of these micro-organisms.

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Rachel E. C. d'Oliveira, Rosana R. Barros, Cláudia R. V. Mendonça, Lúcia M. Teixeira and Angela C. D. Castro

Instituto de Microbiologia, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil

Correspondence: Angela C. D. Castro (acastro@micro.ufrj.br)

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