Colonization by Group B Streptococcus (GBS) is highly prevalent among pregnant women, with rates ranging between 4% and 30%. Vertical transmission during labor is the main source of serious GBS neonatal diseases such as pneumonia and meningitis. In order to prevent such threats, the Centers for Disease Control and Prevention (CDC) recommend that pregnant women should be screened for GBS colonization around the 35th-37th gestational week, and those harboring the microorganism should be submitted to intrapartum antimicrobial prophylaxis. In Brazil, however, no national guidelines regarding this subject exist, and GBS screening during pregnancy is mostly restricted to those patients attending private clinics; hence, GBS occurrence among the pregnant population is still largely unknown. In the present study, we evaluated the occurrence of GBS colonization among 3526 pregnant women between the 35th and 37th gestational week seeking medical attention at a public maternity in Rio de Janeiro State from March 2008 to June 2015. The association of clinical, social and demographic factors with GBS colonization was also investigated by using statistical tools. A single ano-genital specimen was collected from each patient by using a sterile swab. The specimens were initially inoculated in selective Todd-Hewitt broth supplemented with nalidixic acid and gentamicin, and derivative cultures were sub-cultured on 5% sheep blood agar plates. After overnight incubation, β-hemolytic colonies were submitted to conventional identification tests (CAMP test and serological detection of group B polysaccharide). Serotype determination was also performed using serological and molecular methods. GBS colonization was detected in 939 (26.63%) of the 3526 patients evaluated, and this colonization rate was uniform throughout the period of time investigated. Serotypes Ia (39%) and II (20%) were the most frequent, followed by serotypes Ib (11%), V (9%), III (7%), and IV (4%). Presence of vaginal discharge (p=0.0037) was associated with a higher occurrence of GBS colonization. The results indicate the high occurrence of GBS colonization among pregnant women in Rio de Janeiro State, highlighting the need for establishment of strategies to avoid GBS colonization at the moment of labor and, ultimately, prevent the occurrence of GBS neonatal syndromes.