Vaginal & rectal carriage of *Streptococcus agalactiae* in the Czech Republic: incidence, serotypes distribution & susceptibility to antibiotics

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**Background & objectives:** *Streptococcus agalactiae* (group B streptococcus, GBS) is the predominant bacterial agent responsible for invasive perinatal infection. To obtain reliable data on vaginal and rectal carriage of *S. agalactiae* in pregnant women in Czech Republic, and to formulate a prevention programme of neonatal GBS disease for the Czech Republic, women at childbirth were screened for vaginal and anorectal carriage of GBS. The isolates were serotyped and tested for susceptibility to antimicrobials including those recommended for intrapartum prophylaxis.

**Methods:** A total of 586 women at childbirth were screened for GBS carriage in vaginal and anorectal regions using the non-enrichment and selective culture media. The isolates were serotyped by precipitation with antisera raised against various serotypes and antigenic extracts prepared according to Lancefield’s modification. Mueller Hinton agar with 5 per cent defibrinated sheep blood was used for antimicrobial susceptibility testing. MIC values were evaluated according to the NCCLS criteria.

**Results:** Using selective media, GBS was detected in 172 (29.3%) of 586 women screened, vaginal and anorectal colonization was found in 21.7 and 24.4 per cent of them, respectively, concomitant vaginal and anorectal colonization was recorded in 16.5 per cent of the women studied. Serotypes III (33.2%), Ia (22.0%) and V (13.9%) prevailed among 172 isolates tested. All isolates were susceptible to penicillin, ampicillin and cefotaxime. The rates of GBS resistance to tetracycline, erythromycin and clindamycin were 83.9, 3.8 and 3.2 per cent, respectively.

**Interpretation & conclusion:** GBS carriage in pregnant women in the Czech Republic is rather high as compared with that reported in literature. The most frequent serotypes III, Ia and V, identified in GBS-colonized pregnant women in the Czech Republic, were among those predominant in the USA and Western Europe. Our findings confirm uniform susceptibility of GBS isolates from pregnant women to penicillin and other beta-lactam antibiotics tested. Resistance to erythromycin remains low in the Czech Republic.

**Key words** Antimicrobials - colonization - pregnant women - prevention - serotype - *Streptococcus agalactiae* - susceptibility

Since the 1970s *Streptococcus agalactiae* (group B streptococcus, GBS) has been the predominant bacterial agent responsible for invasive perinatal infection. Neonates born to mothers who are colonized at delivery with GBS are at risk of the disease. Though GBS colonization rates vary with ethnic groups, geographic localization and age, they are similar for pregnant and nonpregnant women. Approximately
10 to 30 per cent of pregnant women are colonized with GBS in the vaginal or rectal area. Of all infants born of these women, 1 to 2 per cent will develop early-onset invasive disease (EOD). In an effort to reduce the incidence of EOD, Centers for Disease Control and Prevention (CDC) issued the revised guidelines for the use of intrapartum antibiotic prophylaxis in 2002. These guidelines recommend universal prenatal culture-based screening for vaginal and rectal colonization in all pregnant women at 35-37 weeks' gestation. Positivity for GBS colonization found in this period of gestation is highly predictive of the presence of GBS at time of childbirth. The use of a single swab of the lower vagina and the anorectum cultured into selective media [Todd-Hewitt Broth with either gentamicin (8 µg/ml) or colistin (10µg/ml) plus nalidixic acid (15 µg/ml)] is recommended for optimal recovery of GBS. Penicillin G (or ampicillin) or erythromycin (or clindamycin) for women with serious penicillin allergy are recommended for maternal prophylaxis during labour.

GBS is regarded as uniformly susceptible to penicillin with only a few exceptions, but its resistance to erythromycin and clindamycin differ worldwide with the highest rate in Taiwan and may be associated with certain serotypes, especially type V.

Of the nine currently identified GBS serotypes, five (Ia, Ib, II, III, and V) are prevalent among pregnant women and neonates with EOD in the United States and Western European countries. Types VI and VIII predominate in isolates from Japanese women. Types IV and VII are encountered only rarely. Serotype distribution may have implications for the development of a multivalent anti-GBS vaccine.

To obtain background data on vaginal and rectal carriage subsequent formulation of a prevention programme of early-onset GBS infection in the Czech Republic, women were screened during childbirth for vaginal and anorectal carriage of S. agalactiae. Distribution of serotypes and susceptibility to six antimicrobials including those recommended for intrapartum prophylaxis were also determined in the isolates obtained.

Material & Methods

A total of 586 women at childbirth were screened for GBS carriage in two hospitals (Prague, Ceske Budejovice) of different regions during September 2001 to May 2002. Two vaginal and two anorectal swab specimens from each woman were collected into tubes with Amies medium (Copan, Italy) to be cultured at the National Reference Streptococcus and Enterococcus Laboratory within 36 h in non-enrichment (Brain Heart Infusion Broth BHIB, Oxoid, Unipath, and Columbia Blood Agar, Oxoid Unipath, CBA) and selective (Todd-Hewitt Broth THB, Oxoid Unipath, and CBA, both with CNA supplement, Oxoid, Unipath) media for 48 h at 36±1°C. Selective media were cultured in a 5 per cent CO₂ atmosphere.

S. agalactiae isolates of 172 carriers were serotyped by precipitation reaction in gel and capillary tubes with antisera raised against Ia, Ib, II to VIII, c, R, X and antigenic extracts prepared according to Lancefield’s modification.

Mueller Hinton agar (Oxoid, Unipath) supplemented with 5 per cent defibrinated sheep blood was employed for antimicrobial susceptibility testing. The minimum inhibitory concentrations (MICs) of penicillin, ampicillin, cefotaxime, tetracycline, erythromycin and clindamycin were evaluated according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines.

Results

Between September 2001 and May 2002, 586 women were screened to show an overall GBS colonization rate...
29.3 per cent (172 of 586). Of the 172 GBS carriers, 73.8 per cent were colonized in the vagina and 83.1 per cent in the anorectum. Fig.1 gives percentages positivity for GBS carriage in either vagina or anorectum or both as found by culture in/on non-enrichment and selective media. Among 172 strains isolated, serotype III prevailed widely, followed by serotypes Ia, V and II (Fig.2). As many as 77.3 per cent (133 of 172) of isolates contained a polysaccharide antigen and either c or R protein, 5.2 per cent (9 of 172) of isolates had only either c or R protein. None of the \textit{S. agalactiae} antigens described to date was not found in 1.8 per cent (3 of 172) of isolates (Fig.2). All 172 GBS isolates were susceptible to penicillin, ampicillin and cefotaxime (MIC$_{90}$ 0.063 mg/l) 83.9 per cent isolates were resistant to tetracycline (Table).

All but one of seven erythromycin-resistant isolates were of constitutive macrolide-lincosamide-streptogramin (cMLS) resistance phenotype and one was of M phenotype (MICs of erythromycin and clindamycin were 4 mg/l and 0.25 mg/l, respectively). Five of seven erythromycin-resistant GBS belonged to serotype V.

**Discussion**

In the Czech Republic, prenatal screening and a prevention programme of EOD caused by GBS have been carried out only partially and in a non-standardized way. Prenatal screening is not based on the CDC-recommended criteria$^3$. Latest data needed for formulation of an effective prevention programme are not available. Our data on GBS carriage, susceptibility to antimicrobials and active surveillance of EOD due to GBS$^{14}$ would be useful as background information for nationwide implementation of GBS prenatal screening and formulation of a prevention program in the Czech Republic.

Results of other studies indicate$^{1,2}$ that 10 to 30 per cent of pregnant women were colonized with GBS in the vagina or rectum. GBS carriage rate (29.3%) in pregnant women found in the present study is high as compared with that reported in the literature$^{1,2}$.

The most frequent serotypes III, Ia, V and II identified in GBS-colonized women in the Czech Republic, were similar to those predominant in isolates from pregnant women and neonates with EOD in the USA and Western European countries$^{9,10}$. Types IV and VII rarely found in these countries$^{10}$ were not identified among the Czech
isolates studied and neither was reported type VIII, frequent in Japan\textsuperscript{11}.

GBS isolates with confirmed resistance to penicillin or ampicillin have not been observed to date. The proportion of isolates with \textit{in vitro} resistance to clindamycin and erythromycin has increased since 1996. The prevalence of resistance among invasive GBS isolates ranged from 7 to 25 per cent for erythromycin and from 3 to 15 per cent for clindamycin\textsuperscript{6,15} and might be associated with certain serotypes\textsuperscript{5}. Our findings confirm uniform susceptibility of GBS isolates from pregnant women to penicillin and other beta-lactam antibiotics tested. GBS resistance to erythromycin remains low in the Czech Republic, but needs further surveillance, particularly in invasive GBS isolates. In agreement with the literature data\textsuperscript{5}, serotype V prevailed among the erythromycin resistant strains.

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