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SHORT COMMUNICATION

# Carriage of Group B Streptococci in Pregnant Women from the Region of Krakow and their Antibiotic Resistance in the Years 2008–2012

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Abstract

The aim of the study was a retrospective analysis of the frequency of group B streptococci (*Streptococcus agalactaie*; GBS) carriage in pregnant women from the region of Krakow, together with an analysis of their drug resistance, carried out between 2008–2012. The study included 3363 pregnant women between 35 and 37 weeks of gestation, studied in accordance with the guidelines of the Polish Gynecological Society (2008). A high percentage of pregnant women who are carriers of group B streptococci was demonstrated. Each year covered by the study, it was in the range of 25–30%, with an average value equal to 28%. The results confirm the need for taking swabs from both the vagina and anus, since 15% of GBS-positive patients showed only rectal carriage. High percentage of isolates resistant to erythromycin was detected, which ranged from 22% to 29%, with an average value equal to 25%, as well as a high proportion of isolates resistant to clindamycin being 17–25%, with an average of 20%. The results indicate the need to standardize the methodology of collecting samples for GBS testing and introduce microbiological diagnostic standards in all gynecological and obstetric centers in Poland, in order to carry out a detailed epidemiological analysis in our country.

Key words: *Streptococcus agalactiae*, antibiotic resistance, carriage of group B streptococci, pregnant women

Beta-hemolytic Group B Streptococci (GBS), represented by *Streptococcus agalactiae,* were the main etiological factor for neonatal infections in the USA in the 1970s. These infections usually appear in the form of Early Onset Disease (EOD), which develops in the first seven days of life with clinical manifestations of sepsis and mortality reaching 50%, or in the form of Late Onset Disease (LOD), which develops between the 7$^{th}$ and 90$^{th}$ day of life and usually takes the form of meningitis (Schrag *et al.*, 2002; Verani *et al.*, 2010).

The recorded rapid growth of the number of GBS infections in newborns was the reason behind devising guidelines aimed at newborn infections prevention by the American College of Obstetrics and Gynecology (ACOG) and Centers for Disease Control and Prevention (CDC) in 1996, and a year later by the American Academy of Pediatrics (AAP). CDC recommendations were updated for the first time in 2001 and published in 2002 (Schrag *et al.*, 2002), and also subsequently subjected to evaluation and published once again in 2010 (Verani *et al.*, 2010). Following the example of American scientists, many member countries of the European Union have introduced their own guidelines with the goal of preventing GBS infections in newborns. These include: Italy in 1996, Spain in 1998 and 2003, France in 2001, Germany in 1996 and 2008, Great Britain in 2003, Belgium in 2003, Switzerland in 2007 and the Czech Republic in 2008 (Rodriguez-Granger *et al.,* 2012). In 2008, under the patronage of the Polish Gynecological Society (PTG), a Polish version of the recommendations was devised concerning the detailed instructions in the prophylaxis of GBS infections in newborns (Kotarski *et al.*, 2008).

The acquired experiences indicate that the most effective method of limiting the number of infections in newborns is the introduction of screening of all pregnant women for GBS carriage and, should a positive result be obtained, implementing targeted perinatal antibiotic prophylaxis. It is a well-known fact that the most significant factor predisposing newborns to the development of infection is the presence of *S. agalactiae* in their mother's genital or gastrointestinal tract, from where the bacteria are transmitted to the baby. Therefore, it is advised to carry out microbiological examination of

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the vagina and anus in women between the 35th and 37th week of pregnancy. As GBS colonization is often transitory, predicting on the grounds of a culture performed earlier than 5 weeks before delivery is fallible and recommended only in special cases, i.a. in women with a threat of premature labor or with premature rupture of membranes (PROM). If there is no test result for GBS carriage, the doctor should administer intrapartum chemoprophylaxis to a pregnant woman who has been diagnosed with at least one of the following risk factors of early onset disease: premature labor or PROM (before the 37th week of gestation); delivery at term, in which over 18 hours have passed since the rupture of membranes; developing fever of over 38°C for unknown reasons during delivery; developing urinary tract infection or bacteriuria caused by GBS during the course of the pregnancy; history of delivering a child, who was diagnosed with an infection of *S. agalactiae* etiology (Kotarski *et al.*, 2008; Schrag *et al.*, 2002; Verani *et al.*, 2010).

In Europe, GBS colonization of pregnant women amounts to, according to literature data, from 6.6% in Greece (Tsolia *et al.*, 2003), 7% in Spain (Bayó *et al.*, 2002), 14% in Great Britain (Colbur *et al.*, 2007), 16% in Germany (Brimil *et al.*, 2006), 30% in the Czech Republic (Motlova *et al.*, 2004) to 36% in Denmark (Hansen *et al.*, 2004). In Poland, the percentage of GBS carriage in pregnant women, which is usually presented, differs significantly depending on the examined patient population and the employed method and comes to 4.3% in Lublin (Stupak *et al.*, 2010), 5.13% in Łodz (Serafin *et al.*, 2010), 11.4% in Warsaw (Kociszewska-Najman *et al.*, 2010), 19% in Rzeszow (Krasnianin *et al.*, 2008), 27.8% in Katowice (Romanik *et al.*, 2011) and 30% in Krakow (Brzychczy-Włoch *et al.*, 2012).

Getting to know the frequency of GBS carriage in pregnant women in various populations in Poland and the analysis of their drug resistance, observed over time, is of significant importance in epidemiological research and also in estimating the group of patients requiring the implementation of suitable antibiotic prophylaxis. Unfortunately, Poland is still lacking in sufficient data obtained with the use of uniform standards of microbiological examination assessing the frequency of GBS occurrence among pregnant women and their drug resistance. Furthermore, there is a lack of analyses depicting the dynamics of changes in carriage and drug resistance of *S. agalactiae* over the period of several years of research.

The objective of the work concerned a retrospective analysis of the frequency of GBS (*Streptococcus agalactiae*) carriage in pregnant women from the region of Krakow, as well as the analysis of their drug resistance, carried out in the years 2008–2012.

The testing of GBS carriage in pregnant women was conducted in the period of time from 1st January 2008 to 31st December 2012 on 3363 patients of the Rafal Czerwiakowski Na Siemiradzkiego Hospital in Krakow. The testing was carried out according to the PTG recommendations (Kotarski *et al.*, 2008), upon prior acquisition of a positive opinion from the Bioethics Committee of the Jagiellonian University No. KBET/143/B/2007.

The research material was constituted by two samples taken from each pregnant woman with the use of separate cotton swabs, including a swab from the vagina taken without the use of a speculum and a swab from the anus taken after overcoming the sphincter resistance. The materials were taken between the 35th and 37th week of pregnancy, during a control visit to the gynecologist, and then, within 4 hours, delivered to the Microbiological Diagnostics Lab of the Jagiellonian University Medical College in Amies (bioMerieux) transport medium. Swabs were carried separately to Todd Hewitt Broth with the addition of gentamicin (8 μg/ml) and nalidixic acid (15 μg/ml) (Oxoid) and incubated for 18–24 h at 37°C in aerobic conditions. After preliminary pre-incubation, the materials were cultivated on Columbia Blood Agar (Difco) solid medium with the addition of 5% sheep blood and cultured for 24 h at 37°C in aerobic conditions. *S. agalactiae* species identification was carried out with the use of a SLIDEX STREPTO B (bioMerieux) latex agglutination test, CAMP test and API STREP (bioMerieux) test.

Drug resistance testing was performed for 1623 *S. agalactiae* isolates obtained from all of the positive materials, from both vaginal and anal swabs. The study was performed with the Kirby-Bauer antibiotic testing method using antibiotic discs: penicillin (10 IU), ampicillin (10 μg), clindamycin (2 μg), erythromycin (15 μg), nitrofurantoin (100 μg) and ofloxacin (5 μg) (Oxoid). The results were interpreted according to EUCAST guidelines (EUCAST 2012). There were three macrolide-lincosamide-streptogramin B resistance phenotypes determined for the *S. agalactiae* isolates, *i.e.* constitutive $MLS_B$ resistance phenotype – $cMLS_B$, inducible $MLS_B$ resistance phenotype – $iMLS_B$, and M phenotype.

The analysis of GBS carriage by year, with particular attention to the materials, i.e. anal swabs, was conducted by a $\chi^2$ (chi-square) test. Meanwhile, the carriage in individual months was analyzed in two ways. The first one involved the employment of $\chi^2$ test analysis; the second one, enabling more accurate investigations, consisted in conducting standardization of the fraction of GBS-positive patients for every month, in which the basis for standardization was determined by the average value and the standard deviation from the whole studied period. The dependence of standardized data on the months or seasons was tested using the analysis of variance and Student's *t*-distribution test. Resistance trends in time were examined with the use of Pearson's regres-

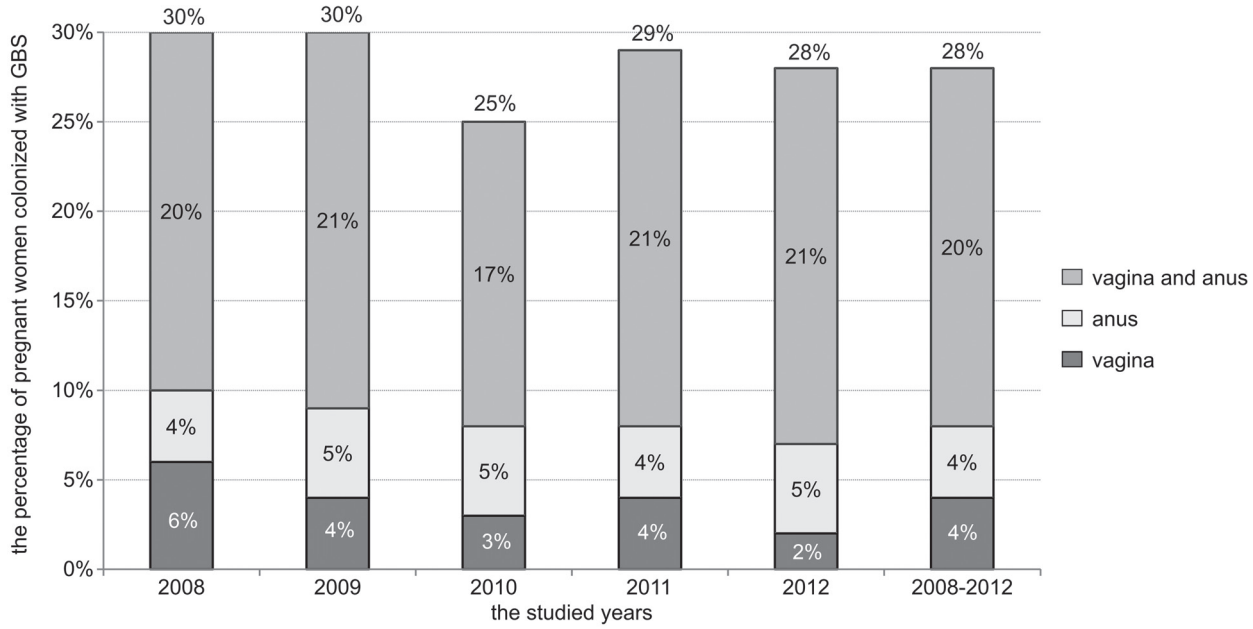


Fig. 1. Frequency of *S. agalactiae* carriage in the vagina, anus and both of them simultaneously in the years 2008–2012.

sion analysis. *P* values of $<0.05$ were considered significant. Analyses were provided with package R 7.02.

In the analyzed period of five years (2008–2012), 3363 pregnant women were included into the study of GBS carriage. They were between their 35$^{th}$ and 37$^{th}$ week of gestation. The number of patients tested in 2008 was 620 (18%), in 2009 – 771 (23%), in 2010 – 802 (24%), in 2011 – 651 (19%), and in 2012 – 519 (16%). The studied group of patients was constituted by women between 18 and 44 years old with a average value age of 30.5. Altogether, 6726 materials, in the form of vaginal and anal swabs collected in pairs from each patient, were subjected to microbiological diagnostics.

The percentage of *S. agalactiae* carriers, determined in the consecutive years of the study, is presented in Figure 1. The place of isolation of streptococci, being either vagina, anus, or both simultaneously, is included. The obtained results point to a high percentage of GBS-colonized pregnant women and remained at a comparable level throughout the subsequent years. GBS carriage in the subsequent years was: in 2008 – 30% (184/620), in 2009 – 30% (233/771), in 2010 – 25% (202/802), in 2011 – 25% (189/651), and in 2012 – 28% (145/519), with the average value for the five-year period of the study reaching 28% (953/3363). It was demonstrated that the year of the study had no significant impact on the frequency of GBS carriage in pregnant women ($\chi^2=6.010$; $p=0.1984$).

The presence of *S. agalactiae* simultaneously in the vagina and anus was determined in 20% (670/3363) of women included into the study, which constituted 70% (670/953) of patients in comparison with the number of GBS-positive patients. The obtained results confirm the necessity to take anal swabs, as in 4% (147/3363) of the patients out of all of the pregnant women included into the research, GBS carriage was only present in anus, which constituted 15% among all the patients who were GBS carriers (147/953). On the basis of statistical analysis, a significantly higher detection of GBS carriage was demonstrated by taking into consideration the material, i.e. anal swabs ($\chi^2=16.365$; $p<0.0001$). Additionally, GBS carriage only in the vagina has been confirmed in 4% (136/3363) of patients, which constitutes 14% (136/953) in the group of GBS-positive patients.

The frequency of *S. agalactiae* carriage in individual months of the year that was analyzed over the period of 5 years encompassed by the research was presented in Figure 2. In selected cases, high divergence of results was obtained for individual months. It can be exemplified by two extreme values; the former being 15% (9/58) of patients colonized with GBS in May 2010 and over a threefold increase in the percentage of GBS-positive patients of 47% (35/47) in August 2009 (data not presented). The average percentage of carriage determined for individual months of the year amounted to: in January – 31%, February – 30%, March – 31%, April – 27%, May – 27%, June – 27%, July – 25%, August – 31%, September – 29%, October – 23%, November – 27%, December – 31%. In order to demonstrate whether there is a relationship between GBS carriage and the individual months, patient attendance was compared in the months encompassed by the research. No significant differences in GBS carriage were found while comparing individual months ($\chi^2=7.721$; $p=0.7380$). Similarly, the analysis of a relationship between the months and the standardized frequency of GBS carriage did not show statistical significance ($f=0.7141$; $p=0.7190$). However, since there were very many categories of

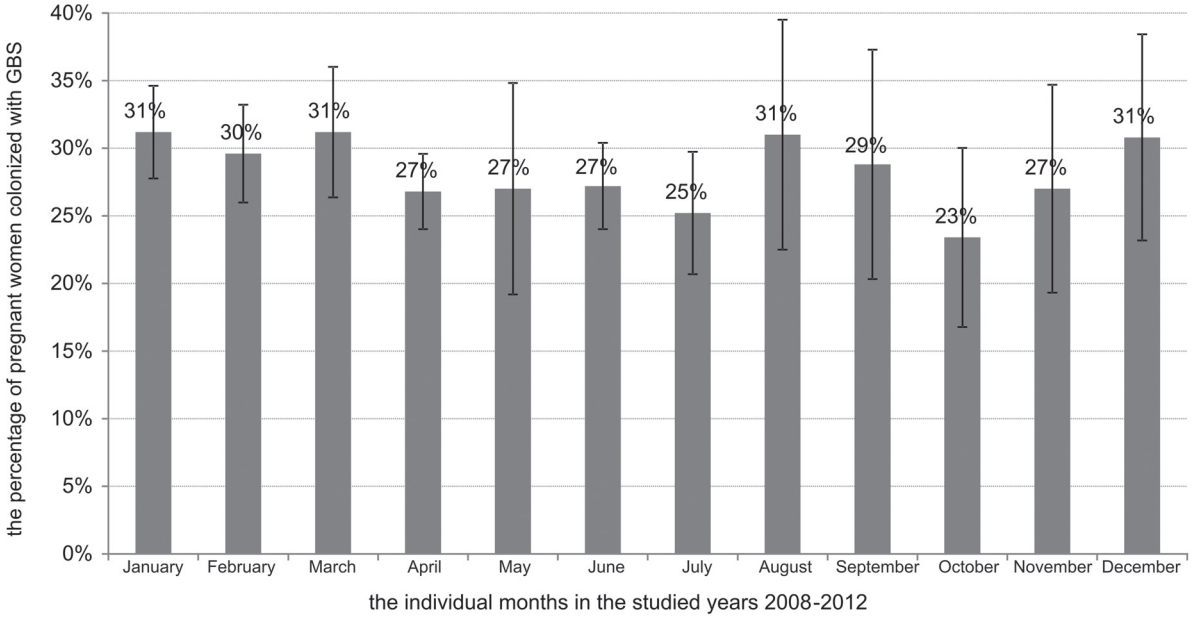


Fig. 2. The average frequency of *S. agalactiae* carriage in individual months in the years 2008–2012.

explanatory variables (months) in the analyzed sample with a relatively small number of measurements (years), showing such a significance is extremely difficult. For this reason, a generalization of the quantitative variable was performed changing it to seasons, what made it possible to demonstrate a difference on the border of statistical significance ($t = 1.940835$; $p = 0.0572$). After removing the outlier, being August 2009 (data not shown), the observed difference was statistically significant for winter (the period from December to March), where the frequency of carriage was higher than with the other months of the year ($t = 2.163241$; $p = 0.0348$).

While analyzing drug resistance, it was determined that all the *S. agalactiae* strains isolated in the period of five years encompassed by the study were sensitive to penicillin, ampicillin, nitrofurantoin, and ofloxacin. In the years 2008–2012, in the studied group of patients, the percentage of strains resistant to erythromycin was 7% (234/3363), while to clindamycin it was 6% (191/3363). Taking into the account the group of GBS-positive patients, the percentage of strains resistant to erythromycin in the years 2008–2012 reached 25% (234/953), in 2008 – 24% (45/184), in 2009 – 22% (51/233), in 2010 – 24% (49/202), in 2011 – 25% (46/189), and in 2012 – 29% (42/145). Similarly, in the case of clindamycin, the percentage of resistant strains in the years 2008–2012 was 20% (191/953), in 2008 – 21% (39/184), 2009 – 17% (39/233), 2010 – 19% (39/202), 2011 – 20% (38/189) and in 2012 – 25% (36/145). In the period of five years encompassed by the study, a resistance trend was determined showing statistically insignificant trend increase ($R^2 = 0.4653$; $f = 2.6115$; $p = 0.2045$). It should be emphasized, though, that the time series involving only five years is incredibly susceptible to artifacts, so obtaining a fully reliable trend assessment would require a long time for analysis.

The percentage of isolates resistant to macrolides and the share of particular resistance phenotypes in the years encompassed by the research were presented in Figure 3. Among resistant isolates, in the years 2008–2012, the domineering one was the constitutive resistance phenotype for macrolides, lincosamides and streptogramins B ($cMLS_B$), constituting 82% (191/234) among all of the resistant GBS isolates, followed by M phenotype 11% (26/234) and inducible phenotype ($iMLS_B$) 7% (17/234).

In 2008, under the patronage of the Polish Gynecological Society (PTG) and on the basis of the CDC recommendations from 2002 (Schrag *et al.*, 2002), a Polish version of the recommendations was devised concerning the detailed recommendations in the prophylaxis of GBS infections in newborns. In accordance with those, a test for GBS carriage in vagina and anus should be performed in pregnant women between the 35$^{th}$ and 37$^{th}$ week of pregnancy (Kotarski *et al.*, 2008). According to the regulation of the Polish Minister of Health from 23$^{rd}$ September 2010 specifying the standards of conduct and medical procedures which accompany providing health services in perinatal care over a woman in the period of her philological pregnancy, childbirth, puerperium, and care over the newborn, it is recommended to perform vaginal vestibule and anal cultures for beta-hemolytic streptococci in all pregnant women between their 33$^{rd}$ and 37$^{th}$ week of pregnancy within the framework of the recommended range of prophylactic services and operations being part of health promoting services, diagnostic tests, and medical consultations (The regulation of the Polish Minister of Health from 23rd September 2010).

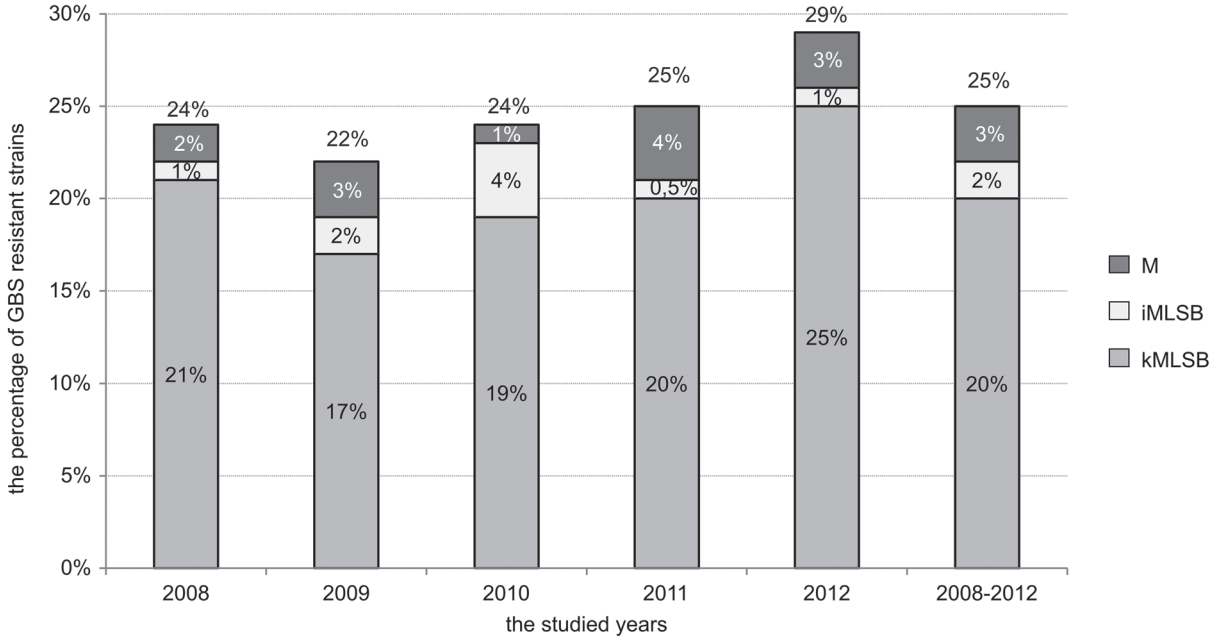


Fig. 3. Percentage of *S. agalactiae* strains with $cMLS_B$, $iMLS_B$ and M resistance phenotypes in the years 2008–2012.

In November 2010, on the basis of the latest epidemiological data and the opinions of experts, CDC together with ACOG and AAP, and also with the American College of Nurse-Midwives, American Academy of Family Physicians as well as American Society for Microbiology, updated the guidelines from 2002 concerning preventing GBS infections in newborns. The most significant changes pertain to: (1) Expanding laboratory methods serving increasing the sensitivity of determining GBS in the studied materials by the employment of chromogenic culture media and the use of commercially-available tests based on nucleic acids amplification. (2) The introduction of screening of pregnant women for determining bacteriuria and establishing the threshold number of GBS amounting to $\geq 10^4$ cfu (colony forming units) for 1 ml urine sample, for which the culture result is deemed positive. (3) Verification of conduct algorithm in the case of women with a threat of premature delivery or premature rupture of fetal membranes. (4) Defining the notion of suitable perinatal antibiotic prophylaxis, which consists in the employment of penicillin (changes in dosage), ampicillin or, additionally, cefazolin at least four hours before labor. (5) The notion of high risk of anaphylaxis was defined and, because of the high percentage of strains resistant to macrolides, erythromycin was withdrawn from perinatal prophylaxis. (6) In addition, the algorithm of conduct with a small newborn threatened with early onset disease (EOD) underwent modification (Verani *et al.,* 2010).

By the employment of PTG recommendations from 2008 concerning the diagnostics of pregnant women for group B streptococci, a high percentage of *S. agalactiae* colonization of pregnant women was confirmed on the basis of the obtained results. The number, in the individual years encompassed by the study (2008–2012), ranged from 25% to 30%, with the average value of 28%. This result brings Poland closer to the countries with the highest percentage of GBS colonization among pregnant women that is described in literature, *i.e.* Slovakia with 30% (Motlova *et al.*, 2004) and Denmark with 36% (Hansen *et al.,* 2004). In comparison with our previous research, from the years 2004–2006, in which carriage, depending on the applied method, was estimated to range between 13% and 17% (Strus *et al.*, 2009) and from the years 2007–2009, in which the participation of GBS-positive patients reached 30% (Brzychczy-Włoch *et al.*, 2012). The current result points to a lingering high percentage of colonized pregnant women residing in the region of Krakow. The observed higher incidence of carriage was most probably connected with the increase in sensitivity of the diagnostic methods and the introduction of standardized microbiological procedures. Hansen *et al.*, 2004, reached similar results upon the introduction of a new differential medium, by which the sensitivity of GBS detection was increased from 15% to 36% (Hansen *et al.*, 2004). The detected high percentage of GBS colonization in the studied group of patients differs significantly from the results obtained by researchers from other centers in our country. For instance, the research of the Warsaw center carried out in the years 2007–2008 recorded GBS colonization in 11.4% of pregnant women (Kociszewska-Najman *et al.*, 2010); similarly, the results of the Łodz center from 2008 indicate only 5.13% carriage in that patient population (Serafin *et al.*, 2010) and the research

performed in 2008 by the Lublin center, in which GBS carriage was demonstrated only in 4.3% of women (Stupak *et al.*, 2010). The low percentage of colonization described by the authors of the quoted studies might have been related to different geographical areas and methodology of the conducted research. For instance, in the research carried out in Lublin, the studied material was constituted only by vaginal swabs and there is a lack of a description of culture methodology and diagnostics of group B streptococci (Stupak *et al.*, 2010). Worth noting to the special resemblance of our results to the data coming from Katowice, where percentage of GBS colonization in pregnant women was 27.8% (Romanik *et al.*, 2011).

It is worthwhile mentioning that the standardization of microbiological diagnostics with the aim of determining GBS carriage in women is of fundamental importance in correct diagnostics of pregnant women. In accordance with CDC, the lack of preliminary material preincubation in a selective medium decreases method sensitivity even by 50% (Schrag *et al.*, 2002). Likewise, collecting swabs only from the vagina or only from the anus influences obtaining false-negative results in as many as 30% of patients (Brimil *et al.*, 2006). The statistically significant results confirm the necessity to take anal swabs in parallel with vaginal swabs, as 15% of patients displayed carriage only in anus. Hence, the omission of the diagnostic material constituted by anal swabs has an impact on obtaining false-negative results in the amount of as much as 15%. Brimil *et al.*, 2006, obtained similar results, which prove the fact that taking only vaginal swabs results in getting false-negative results in 24% of cases (Brimil *et al.*, 2006). Summing up, according to the current PTG recommendations from 2008, there are several factors significantly influencing a correct test result for GBS carriage, *i.e.* the type of material collected, it is required to be a vaginal and an anal swab, which, after their collection, can be put together into one test tube; employing appropriate transport media, *e.g.* Amies medium; preliminary 18–24 h material preincubation, often skipped in many labs, which can be carried out, for example, on Todd Hewitt Broth with an addition of suitable antibiotics hampering the proliferation of other bacteria; isolation of all colonies characteristic of streptococci from the medium with blood, even the ones which do not possess beta hemolysis, since there is a small proportion of *S. agalactiae* strains that do not display this feature (Kotarski *et al.*, 2008; Schrag *et al.*, 2002; Verani *et al.*, 2010).

A detailed analysis of the dynamics of change for individual months of the calendar year pointed to a statistically significant increase in carriage in winter months, in comparison to the remaining parts of the year. Ma *et al.*, 2012, in the research concerning pregnant women from Taiwan, demonstrated an increase in frequency of isolation of GBS strains representing hipervirulent ST-17 clone in winter months (Ma *et al.*, 2012). On the other hand, the study carried out by Dadvand *et al.*, 2011, indicates a statistically significant relation between the frequency of GBS carriage and August, the warmest and most humid month in Spain (Dadvand *et al.*, 2011). The obtained result is difficult to discuss as there are no publications describing analyses of this kind carried out on a similar sample group residing in a geographical area comparable as regards the climate.

Even though penicillins have been used for many years now, most of the *S. agalactiae* strains are still sensitive to this antibiotic (Schrag *et al.*, 2002; Verani *et al.*, 2010). However, the first strains of GBS with reduced penicillin susceptibility (PRGBS) were characterized in Japan (Kimura *et al.*, 2008). Our research results confirm the sensitivity to penicillin and ampicillin for all of the GBS strains isolated in the period of five years encompassed by the study. However, in recent years, GBS strains' resistance to macrolides, lincosamides and streptogramins B is more and more often described. Uh *et al.*, 2005, demonstrated significant increase in the frequency of isolation of GBS strains resistant to erythromycin, from 0% in the 1990s to 41% in 2002, and to clindamycin, from 0% to 48% in the same periods (Uh *et al.*, 2005). In Europe, the percentage of GBS strains resistant to erythromycin is from 11% in Germany (Schoening *et al.*, 2005), 14% in Spain (Gherardi *et al.*, 2007), 16% in Italy (Gherardi *et al.*, 2007), 21% in France (De Mouy *et al.*, 2001), to 22% in Turkey (Acikgoz *et al.*, 2004). The high percentage of *S. agalactiae* strains resistant to erythromycin reaching 25% and the high proportion of isolates resistant to clindamycin reaching 20%, were recorded by us, confirm the worldwide tendencies, indicating increase in GBS drug resistance. Two mechanisms are responsible for the resistance of streptococci to macrolides. The first one consists in active removal (pumping out) of the antibiotic from a bacterial cell. This mechanism is marked with phenotype M, coded by *mef* gene and determines resistance to 14- and 15-membered macrolides, *i.e.* erythromycin, clarithromycin, roxithromycin and azithromycin, but resistance to 16-membered macrolides, *e.g.* spiramycin and clindamycin. The second type of resistance stems from methylation in place of the target activity in the ribosome and conditions the resistance to all the macrolides, lincosamides, and streptogramins B, which is described with $MLS_B$ phenotype of constitutive or of inducible character; however, clinically, it indicates a lack of effectiveness of all of those drugs (cross-resistance) (Acikgoz *et al.*, 2004; Uh *et al.*, 2005). In the isolate pool studied by our team, the constitutive phenotype was the dominant one (82%), followed by phenotype M (11%) and the inducible phenotype (7%),

at the same time, the share of particular phenotypes was different in the individual years of the research.

To sum up, in the light of literature data from our country, often making use of different methods for diagnosing GBS from the recommended ones and also using the standards modified in 2010 by CDC, it is necessary to consider an update of the 2008 PTG recommendations and publishing them again, following the example of other countries, under the patronage of all of the Polish societies, directly related to the problem of newborn infection prophylaxis.

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