Slovenian Medical Journal

Prevention of early invasive infections with group B streptococci in the newborn – microbiological methods for detection of collonization before and at birth

Preprečevanja zgodnjih invazivnih okužb novorojenčka zaradi streptokoka skupine B – mikrobiološke metode za ugotavljanje nosilstva pred in ob porodu

Ajda Kovačič,[1] Gal Kranjc,[1] Miha Lučovnik,[1,2] Petra Vovko,[3] Samo Jeverica[3]

Abstract

At the end of last year, the Health Council of the Republic of Slovenia adopted a programme for the prevention of early invasive neonatal infections caused by group B streptococci, which includes universal screening of pregnant women between the 35th and 37th week of pregnancy. In this article, we provide an overview of the different diagnostic modalities for screening for collonization and the factors that significantly influence the success of screening, both in gynaecological practise and in the microbiology laboratory. We instruct the reader on the proper collection and transport of specimens. We also present the chosen testing strategy, using a combination of enriched culture and molecular testing, and provide the reader with a list of registered molecular tests suitable for screening. In the last part of the article, we discuss the importance of the hypervirulent clone CC -17, which causes most invasive neonatal infections in Slovenia, and the methods by which it can be detected.

Izvleček

Ob koncu lanskega leta je Zdravstveni svet Republike Slovenije potrdil program preprečevanja zgodnjih invazivnih okužb novorojenčkov, povzročenih s streptokokom skupine B, ki vključuje univerzalno presejanje nosečnic v 35.–37. tednu nosečnosti. V prispevku smo pregledali različne diagnostične metode presejanja in dejavnike, ki pomembno vplivajo na njihovo uspešnost tako v ginekološki ambulanti kakor tudi v mikrobiološkem laboratoriju. Bralca opozarjamo na pravilnost odvzema kužnine, mu predstavimo izbrano strategijo testiranja s kombinacijo obogatene kulture in molekularnega testiranja ter mu ponujamo seznam registriranih molekularnih testov, primernih za testiranje. V zadnjem delu prispevka razpravljamo o pomenu hipervirulentnega klona CC-17, ki povzroča večino invazivnih okužb novorojenčkov v Sloveniji, in o metodah, s katerimi ga prepoznamo.

[1] Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
[2] Division of Gynaecology, University Medical Centre Ljubljana, Ljubljana, Slovenia
[3] National Laboratory of Health, Environment and Food, Maribor, Slovenia

Correspondence / Korespondenca: Samo Jeverica, e: samo.jeverica@nlzoh.si

Key words: pregnancy; neonatal infections; streptococcus agalactiae; diagnostic methods; molecular detection; screening

Ključne besede: nosečnost; neonatalne okužbe; Streptococcus agalactiae; diagnostične metode; molekularna diagnostika; presejanje

Received / Prispelo: 20. 12. 2020 | **Accepted / Sprejeto:** 5. 4. 2021

Cite as / Citirajte kot: Kovačič A, Kranjc G, Lučovnik M, Vovko P, Jeverica S. Prevention of early invasive infections with group B streptococci in the newborn – microbiological methods for detection of collonization before and at birth. Zdrav Vestn. 2022;91(7–8):309–18. **DOI:** https://doi.org/10.6016/ZdravVestn.3202

1 Introduction

The proportion of invasive infections (sepsis, meningitis and pneumonia) in the morbidity and mortality of newborns and children up to the third month of age is significant (1). In Slovenia, about a fifth of neonatal deaths are caused by invasive infections (2). As in the developed world, the most common causative agent in Slovenia is the group B streptococcus (GBS), causing up to 50% of invasive infections (2,3). These are classified as early-onset (0–7 days of age) and late-onset (8–90 days of age). Early neonatal GBS infections result from vertical transmission of the bacteria from the mother at the time of delivery. Their incidence in Slovenia is >30% higher than the global and European estimates, and amounts to 0.53/1,000 births (3,4). With advances in treatment, mortality has decreased below 10%, but a large proportion of children can have permanent sequelae in mental and motor development (20–30%), representing a significant disease and economic burden on society (4-6).

The proportion of Slovenian pregnant women who are carriers of GBS in the intestine or vagina was determined in two studies, and was found to be 17% and 23% respectively (7,8). The transmission of bacteria from mother to child during vaginal delivery occurs in approximately half of pregnant women, and invasive neonatal infection occurs in 1–2% (9). In the 1980s, it was found that early invasive infections can be effectively (>90%) prevented by intrapartum antibiotic prophylaxis, in which the mother receives an antibiotic intravenously during childbirth (9).

Pregnant women can be selected for antibiotic prophylaxis in three ways: a) by the presence of perinatal risk factors (premature birth, prolonged rupture of membranes, fever, presence of GBS in the urine during pregnancy or neonatal GBS infection during a previous pregnancy), b) in the case of previously established GBS colonization in the third trimester of pregnancy, typically between the 35th and 37th week, or c) in the case of established perinatal GBS colonization of the mother (10). Numerous studies have established that screening between the 35th and 37th weeks of pregnancy is more effective than simply identifying risk factors (11,12). In a recent national study, perinatal risk factors were absent in 52% of affected children, which confirms the insufficiency of prevention based solely on this type of risk assessment (4).

The Health Council of the Republic of Slovenia recently adopted a proposal to introduce universal screening for GBS in the 35th to 37th week of pregnancy. In this paper, we present the natural history of GBS colonization and both new and already established methods for determining GBS colonization in pregnant women.

2 The natural history of GBS colonization

Infection and colonization of the mother are necessary prerequisites for the transmission of bacteria to the child in early-onset infections. It is assumed that the mother is colonized via the faecal-oral route from another person (close contact, including sexual contact), from the environment (due to poor hygiene) or through food (dairy products), and then from the intestine, GBS colonization spreads to different parts of the body, particularly to the urinary and reproductive tracts. Concurrent colonization of several body regions is frequently present (13-15). The dynamics of colonization are probably more complex than is commonly believed. In a Danish study, pregnant women were monitored during and after pregnancy. They found that just over half of the women (53%) were not colonized at the time of the study. The rest were either permanent (28%) or intermittent carriers (19%). The population of bacteria was genetically homogeneous in each woman and the bacterial strain's genotype did not change over time. They concluded that the GBS proportion of the intestinal microbiota at the time of sampling is important for their successful detection (16).

Colonization status in the late third trimester of pregnancy is a predictive factor of actual colonization at the time of delivery. As the time from testing to delivery increases, the predictive value of such testing decreases. The negative predictive value of a sample taken <5 weeks before delivery is a satisfactory at 95–98%, but before this period it drops rather steeply to 80% (17,18). The poorer negative predictive value is the reason that in environments that perform screening, most early-onset invasive neonatal infections are observed in pregnant women with a negative antenatal screening test result. Such cases may be the result of a false-negative test or new colonization of the pregnant woman during the period from testing to delivery (11).

In the latest guidelines, the American College of Obstetricians and Gynaecologists (ACOG) has changed its recommendation for taking vaginal-rectal cultures in the period >5 weeks before delivery to determine GBS carrier status due to the poorer negative predictive value

of testing. Now, instead of performing screening after 35 0/7 weeks of pregnancy, they recommend performing screening between 36 0/7 and 37 6/7 weeks of pregnancy (19). In this way, the window until delivery at term was shortened to five weeks (36 0/7 to 41 0/7). If the delivery takes place >5 weeks after screening, repeat GBS screening is reasonable.

The opinion of the article's authors is that such a solution eliminates some shortcomings in determining antenatal colonization. However, we would like to point out that after the 37th week of pregnancy, the risk that delivery will start before we receive the screening results begins to increase. Given that our system of antenatal care does not provide for weekly examinations until the 37th week, we suggest that our recommendation remains unchanged for now and for screening to be performed between the 35th and 37th weeks of pregnancy; it should be noted that screening between the 36th and 37th weeks is more appropriate, when possible. In the future, based on data from individual periods, we will change the recommendation accordingly.

3 Antenatal colonization detection

In the following text, we present the basic microbiological method for determining antenatal colonization between the 35th and 37th week of pregnancy, i.e. enrichment culture, and list some of the more important improvements that increase the detection method's sensitivity.

3.1 Enrichment culture

Use of an enrichment culture is the standard for determining GBS colonization; one or two vaginal-rectal specimens are first incubated in a selective liquid broth (enrichment). After 16–24 hours, the incubated broth is subcultured to an appropriate agar plate. For enrichment, the Todd-Hewitt broth supplemented with gentamicin (8 µg/ml) and nalidixic acid (15 µg/ml) or the Lim broth with colistin (10 µg/ml) and nalidixic acid (15 µg/ml) are used. The antibiotics inhibit the growth of Gram-negative bacteria. After overnight incubation, the enrichment broth is subcultured to one of the agar plates used to detect GBS colonies, such as blood agar, blood agar supplemented with colistin and nalidixic acid (CNA agar) or one of the specialized commercial chromogenic agars. The subcultured agar plates should be incubated to an additional 48 hours (12,20).

The identification of typical GBS colonies, characterized by beta haemolysis, on solid culture media has recently been mostly performed by MALDI-TOF (Matrix Assisted Laser Desorption/Ionisation Time of Flight) mass spectrometry. It can also be performed using one of the older identification methods, either with latex agglutination or biochemical tests. Bacterial cultivation is currently the only way to test the antimicrobial susceptibility of GBS to antibiotics, which is particularly important in cases of beta-lactam allergy and for resistance control (12,20).

Nonhaemolytic and nonpigmented (NH/NP) strains, which appear in 2–5% of positive samples and can just as easily cause invasive infections, are a problem in GBS screening using bacterial cultures (21). The absence of colony haemolysis and pigmentation mainly affects phenotypic identification, which requires more staff experience and a greater number of additional tests used for identification, and can be the cause of false-negative results when using enrichment broth. Similarly, the competitive growth of *Enterococcus faecalis*, which is a frequent intestinal colonizer and grows together with streptococcus in enrichment broth, can inhibit GBS growth on selective media (22).

The following two factors play an important role in the successful GBS detection in an enriched culture: a) using selective enrichment broth in b) collecting combined vaginal-rectal specimens (Figure 1). Using enrichment broth has a significantly higher sensitivity than using only direct agar plating. It is estimated that without enrichment broth, up to 50% of tests are falsely negative (12,23). Furthermore, collecting combined vaginal-rectal specimens is also very important. This is primarily a reflection of the natural colonization process, in which the intestine is colonized first, followed by bacterial growth and vaginal colonization. In most studies, GBS was more frequently found in the rectum than the vagina, up to a ratio of 2:1 (24,25). Combined specimen sampling from both anatomical sites improves detection rates by approximately 30% (23).

We must emphasize that the gynaecologist is responsible for specimen collection and that suboptimal specimen collection, as is often the case in microbiology, has a significant impact on the validity of the results. Therefore, the data from the Slovenian 2016 study, in which only a minority (17%) of specimens were collected correctly, is worrying (8).

When using enrichment culture, live bacterial cells must be transported to the laboratory. Therefore, high-quality transport systems consisting of swabs and a transport medium are important. We have traditionally used cotton-tipped swabs and a semi-solid transport medium. Today, flocked swabs with an improved

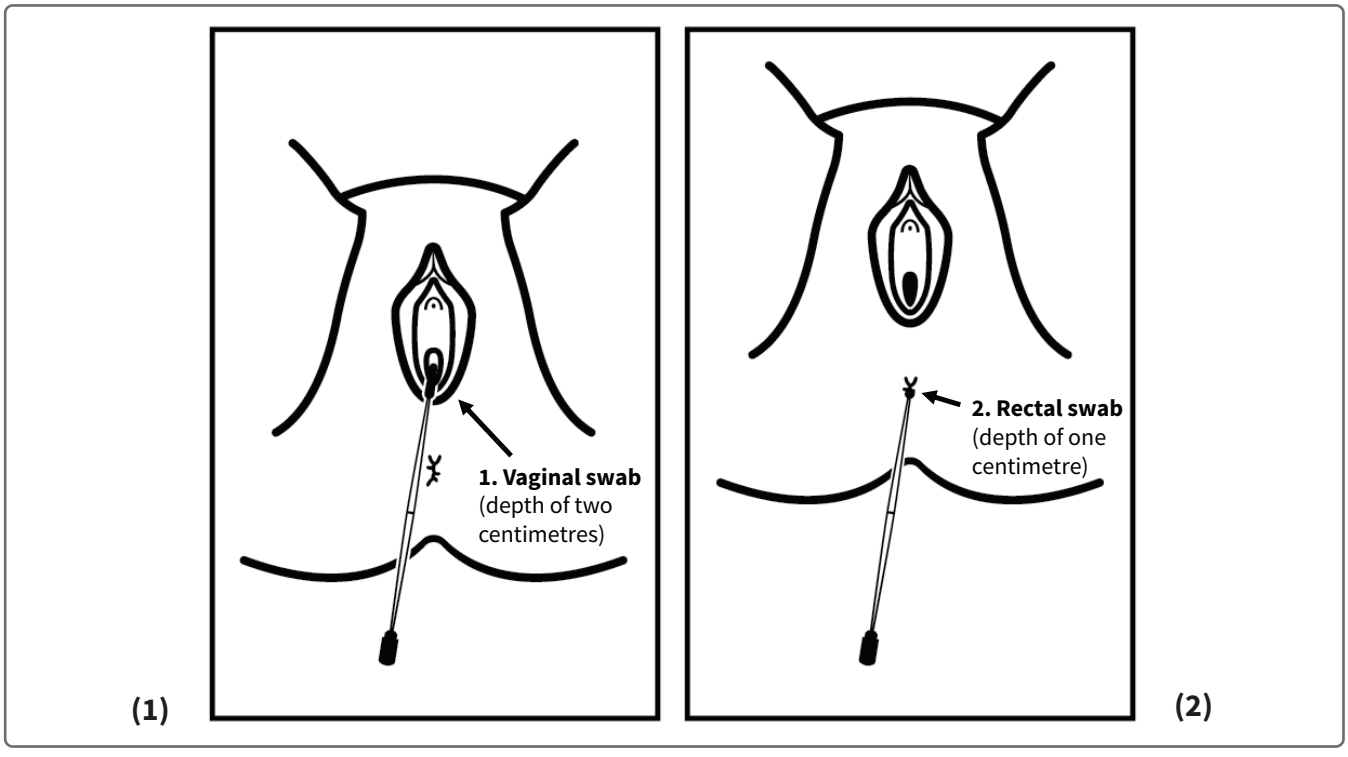

Figure 1: Specimen collection instructions for determining GBS colonization of pregnant women. Adapted from the Centres for Disease Control and Prevention (CDC) instructions (12). First, a vaginal specimen at the depth of two centimetres is collected (1), followed by rectal specimen collection (with the same swab) (2).

Emphasis: sample collection and transport.
For vaginal-rectal specimen collection, a swab with a transport medium (e.g. Stuart, Amies) is required, which is first inserted into the lower third of the vagina (2 cm deep) and then into distal rectum (1 cm deep). The swab is rotated in both anatomical places for about five seconds to allow adequate specimen collection. The specimen is then placed in a transport medium and sent to the microbiological laboratory within 48 hours, preferably as soon as possible. Specimen transport takes place at room temperature. When the specimen cannot be sent to the laboratory on the same day, it should be kept in the refrigerator (+ 4 °C) for up to four days (Figure 1) (12).

composition and topography of the synthetic fibre tip are available, preventing bacterial containment in the tip and improving their release into the transport media (26). Bacteria remain viable in transport media for at least 48 hours at room temperature. When specimens cannot be sent to the laboratory immediately, they can be stored in a refrigerator (4 °C) for up to four days (12). Some manufacturers combine swabs with enrichment broth (THBS, Lim, Carrot), which means that incubation in the laboratory can start without additional specimen manipulation.

3.2 Improving enrichment culture

3.2.1 Chromogenic media

In this paper, chromogenic media are defined as all media in which positivity is equated with a change in the colour of either the media or bacterial colony. This category includes both liquid chromogenic media used in the enrichment phase (Carrot broth) and solid chromogenic media used for direct plating or plating after enrichment. Based on the mechanism of the colour reaction development, we distinguish between chromogenic agars and Granada-type media.

In recent years, there has been rapid expansion in the availability and use of chromogenic media for the detection of various pathogenic bacteria, including GBS (22). Chromogenic agars contain enzyme substrates specific for individual bacteria linked to chromogenic molecules (indoxyl compounds), which are released into the environment after an enzymatic reaction, leading to precipitation within and typical staining of bacterial colonies (27). Unlike blood agar and Granada media, only chromogenic agars can detect NH/NP GBS strains. They are characteristically cultured under aerobic conditions,

which are optimal for substrate degradation and colour reaction development. Since chromogenic compounds degrade in light, they must be stored in the dark (22).

Chromogenic agars can be used both for direct plating and plating after enrichment. The ability of chromogenic agars to stain NH/NP strains is the basic reason for their improved sensitivity. Compared to other culture media, approximately 5% more positive cultures are detected, reflecting the proportion of NH/NP strains in the population (28). However, colour change detection is not always straightforward. GBS colonies can stain poorly, particularly when commensal flora is abundant and significantly exceeds the GBS proportion. On the other side of the spectrum, colonies of bacteria related to GBS can also be stained, including other streptococci or even some enterococci and staphylococci. An experienced examiner will recognize such non-specific colonies macroscopically, but the authors advise caution and recommend controlling the identification of all stained and suspicious colonies with the MALDI-TOF mass spectrometry method, if possible (22).

Many chromogenic media from different manufacturers are available on the market, including: bioMérieux (ChomID Strepto B), Bio-Rad (StreptoB Select), Oxoid (Brilliance GBS) and others. If swabs are inoculated directly onto them and incubated for one day, a sensitivity of about 85% (77–96%) is achieved. If the swabs are first inoculated into liquid enrichment broth and then into chromogenic media, which are incubated for an additional two days, the sensitivity approaches 100% (93–100%) (29-34). Of course, it should be emphasized that the sensitivity of 100% in this case reflects the fact that this method was used as the gold standard.

The second type of chromogenic media (Granada-type) are media that stimulate natural pigment formation in the vast majority of GBS strains. This property is linked to the formation of haemolysins, so NH/NP strains are not detected using these culture media. Pigment formation is enhanced under anaerobic conditions (20). Compared to chromogenic agars, the sensitivity of Granada-type media is expectedly worse and is around 95% after enrichment (34). Pigment formation is also used in some liquid enrichment media (Carrot broth, liquid biphasic Granada medium), but their sensitivity is worse than established liquid media. Their popularity is mainly attributed to the high specificity of the orange pigment and the fact that stained liquid cultures do not need additional confirmation of GBS detection. The declared sensitivity of one of the registered liquid culture media (Strep B Carrot Broth) is 88%. In the absence of visual colour production, the broth should be subcultured to solid medium which is observed for 48 hours for GBS growth (35).

3.2.2 Molecular assays

The use of molecular methods has greatly changed diagnostic microbiology. In the field of determining GBS colonization of pregnant women, many molecular tests have been developed, among which, at the time of writing this article, 12 were registered with the US Food and Drug Administration (FDA) (reviewed on 21.11. 2020). Among them, 11 tests are intended for GBS confirmation after prior enrichment, and only one is for testing directly from the specimens taken during delivery (Table 1).

GBS detection by molecular assays after overnight enrichment in liquid culture media greatly improves sensitivity and shortens testing time. With such testing methods, 20–40% more colonized pregnant women are detected on average compared to the standard method described in the guidelines of the American Centres for Disease Control and Prevention (CDC) (12,28,36-41). Such testing is also faster and can be completed within 24 hours, i.e. 1–2 days faster than the standard method. Because of the above, some believe that the combination of enrichment and molecular GBS detection should be considered as the new gold standard for screening. (22,41). This type of testing was also confirmed in the Slovenian programme for the prevention of early-onset invasive neonatal infections caused by GBS. Briefly, after admission to the laboratory, combined vaginal-rectal specimens are first inoculated in THBS enrichment broth for overnight incubation (16–18 hours). The next day, the broth is tested for GBS with one of the registered molecular tests according to the test manufacturer's instructions. There is an increasing number of commercial molecular test manufacturers for GBS detection on the market, which will undoubtedly reduce prices and increase the availability of this type of testing method (Table 1).

3.2.3 GBS antigen detection

In the past, a number of antigenic tests for urinary and vaginal GBS detection were developed, but regardless of the detection method (latex agglutination, enzyme immunoassay, optical immunoassay), they proved to be insufficiently sensitive when used directly on the collected specimens. The bacterial burden and the associated number of antigens can be very small during colonization. Compared to enrichment, the sensitivity of antigen

Table 1: Molecular tests that are registered with the US Food and Drug Administration (FDA).

Molecular test	Registration year	Testing method	Method	Target (gene)	Sensitivity[1] % (95% CI)	Specificity[1] % (95% CI)
Xpert GBS*	2006	Direct	rtPCR	*cfb*	89 (83-93)	97 (95-98)
Xpert GBS LB	2012	After enrichment	rtPCR	*cfb*	99 (96-100)	92 (90-94)
BD Max GBS	2012	After enrichment	rtPCR	*cfb*	95 (90–98)	97 (95–98)
Alethia GBS	2012	After enrichment	LAMP	ND	99 (97-100)	93 (92-95)
AmpliVue GBS	2013	After enrichment	HDA	*atoB*	100 (97-100)	93 (91-94)
Aries GBS	2016	After enrichment	rtPCR	*cfb*	96 (91-98)	91 (89-94)
Great Basin Portrait GBS	2016	After enrichment	rtPCR	*cfb*	98 (93-99)	96 (94-98)
Solana GBS	2017	After enrichment	HDA	*atoB*	100 (98-100)	96 (94-97)
GenePOC GBS LB	2017	After enrichment	rtPCR	*cfb*	96 (92-98)	96 (94-97)
NeuMoDx GBS	2018	After enrichment	rtPCR	*pcsB*	97 (94-98)	96 (95-97)
Simplexa GBS	2018	After enrichment	rtPCR	*cfb*	97 (92-99)	96 (93-98)
Panther Fusion GBS	2020	After enrichment	rtPCR	*cfb,sip*	100 (98-100)	97 (95-98)

Legend: rtPCR – real-time polimerase chain reaction; LAMP – loop-mediated isotermal amplification; HDA – helicase-dependant amplification; ND – unknown; GBS – I. group B streptococcus.
[1] Sensitivity and specificity as listed in FDA documentation.
* The only diagnostic test that is currently also registered for intrapartum screening and has a lower sensitivity compared to the others.

tests is 15–74%, so most professional associations do not recommend them for detecting colonization (12,22,42).

Due to the high specificity of these types of tests, nevertheless, and their ease of use, there have been attempts to use antigen tests after enrichment, particularly recently. Such use of rapid antigen tests could possibly become comparable to the molecular GBS detection after enrichment (43,44,45).

4 Intrapartum GBS screening

The development of rapid and simple molecular methods which do not require an advanced molecular laboratory and experienced technical staff has made it possible to detect colonization in pregnant women directly upon admission to the maternity ward or at labour onset. Such a method of testing could improve some of the shortcomings of antenatal testing, namely: a) the problem of premature birth before the 35th week of pregnancy, b) the problem of intermittent colonization, in which the carriage status can change from the time of testing to delivery, c) the logistical problem of testing between the 35th and 37th weeks of pregnancy, which could be a problem particularly in less developed countries, and ultimately, d) the problem of unnecessary antibiotic prophylaxis in pregnant women who are not demonstrably colonized with GBS during childbirth (42).

However, intrapartum testing also has some disadvantages. The existing rapid molecular tests are completed at the fastest in 1–2 hours from specimen collection. In many cases, this is not fast enough for all pregnant women, some of whom may give birth before the test is complete (46). Subsequently, many studies have shown that the sensitivity of molecular tests without prior enrichment is 10–20% lower than with tests with prior enrichment (47-49). In this way, with a less sensitive test, colonized pregnant women could be missed and therefore wouldn't receive antibiotic prophylaxis. In most studies to date, it has been shown that technical problems appear in approximately 10% of test, which then requires repeat testing; again, it becomes an issue of time. Finally, at the time of writing, there is only one registered test used for this purpose (Xpert GBS).

Because of all this, intrapartum screening is not

recommended by any professional association for the time being. On the contrary, such testing is expressly discouraged by the American Society for Microbiology (ASM), as testing without enrichment is not sufficiently sensitive and has a low negative predictive value (50). Nevertheless, recent individual pilot studies have demonstrated both the advantages and disadvantages of intrapartum screening (51-53). In any case, it is necessary to regularly monitor innovations in the field of molecular diagnostics, which in the future could be the basis for intrapartum screening; if possible, such testing in local environment is preferable.

5 Hypervirulent GBS clone (serotype III, clonal complex 17)

The polysaccharide capsule is the most important GBS virulence factor. According to the capsular polysaccharide, GBS is divided into 10 different serotypes (Ia, Ib, II-IX), which differ from each other antigenically and structurally (54). In Europe, the most common serotypes are Ia, II, III and V, with serotype III causing most invasive infections (3). Colonization success and infection development are also related to the presence of other virulence factors, among which various adhesive molecules and structures that allow adhesion either to the epithelial surface (pili, HvgA) or molecules of the extracellular matrix (FbsA-C, Srr1-2, Lmb, ScbP and others) are very important (55).

GBS is a commensal bacterium. It is therefore surprising that, at the genomic level, most of the human colonization and clinical isolates belong to only five clonal complexes (CC – CC-1, CC-12, CC-17, CC-19 and CC-23). Among them, the clonal complex CC-17 is considered to be hypervirulent as it causes the vast majority (>80%) of late-onset invasive infections and dominates among the causative agents (50%) of early-onset invasive infections. Most strains of the CC-17 clone belong to the capsular serotype III and have the hypervirulent adhesin A (*hvgA*) gene. HvgA contributes to adhesion to intestinal epithelial cells, vascular endothelium and choroid plexus cells in the brain (55).

From an evolutionary point of view, the main event in the formation of such a homogeneous GBS population structure is considered to be the beginning of medical use of tetracycline in 1948, and the acquisition of the tetracycline resistance gene (*tetM*) (56). These two events initiated the selection of a very limited number of human adapted tetracycline resistant GBS clones. Coinciding with this was the increase in GBS infections in the 1960s, including invasive infections of pregnant women and neonates. In several longitudinal studies that monitor the incidence of invasive neonatal infections at the clonal level, it is concluded that it is the replacement of clones within the GBS population, and particularly the emergence and spread of the hypervirulent CC-17 clone, that is the reason for the reduced effectiveness of programmes to prevent neonatal sepsis (57).

In Slovenia, we have recently genomically characterized all available invasive neonatal GBS isolates and compared them with colonization isolates. As expected, we found that serotype III, CC-17 isolates predominated

Table 2: NMicrobiological testing methods and their basic characteristics for determining colonization of pregnant women. Adapted from Rosa-Fraile M., et al., 2017 (22).

Type of test	Duration	Relative sensitivity[1]	Relative specificity	Antibiotic susceptibility testing	Relative cost[2] (€)
Enrichment culture*	48-72 h	90-95%	90-100%	Yes	≈15-20
Molecular (after enrichment) *	24 h	95-100%	95-100%	Conditional[#]	≈50-60
Molecular (direct)**	1-2 h	85-95%	95-100%	No	≈45-55
Antigen (direct)	Up to 1 h	15-75%	up to 75%	No	≈10

[1] In most comparative studies, the enriched culture (CDC-recommended method) was considered as the gold standard (11). In studies comparing molecular tests after enrichment, such testing detected 20-40% more colonized pregnant women (see text).
[2] The indicated prices are approximations.
[#] With concurrent direct chromogenic agar plating, antibiotic susceptibility testing can normally be performed.
* Suitable for prenatal testing (35th to 37th weeks of pregnancy).
** Suitable for intrapartum screening. In most studies, such testing was about 10% less sensitive.

among invasive (67%) and non-invasive isolates (33%). Their predominance was particularly high in late-onset invasive infections (81%) compared to early-onset infections (48%) (58).

From a microbiological point of view, in addition to traditional typing methods based on sequencing part of the genome, there are also some simpler and faster methods for determining the CC-17 clone. On the one hand, there is the specific presence of the *hvgA* gene, which can be used as a genetic and diagnostic marker of infection with this clone (59). On the other hand, CC-17 also differs from other GBS clones in the presence of specific peaks in MALDI-TOF mass spectrometry, a method that is commonly used to identify bacteria in recent times (60). Whether hypervirulent CC-17 clone detection could contribute to disease prognosis is difficult to say for the time being, as no such clinical data exists.

6 Conclusion

With the adoption of the GBS screening programme in pregnant women in 2020, much has been achieved to reduce the incidence of early-onset invasive neonatal GBS infections, which is currently higher in Slovenia than in comparable countries. We decided to use the currently most sensitive diagnostic methodology, i.e. combinations of enrichment and molecular testing (Table 2). In this way, we combined the advantages of both methods, achieved higher sensitivity, enabled the possibility of determining antibiotic resistance, and shortened the diagnostic procedure duration; higher screening sensitivity and specificity was achieved with molecular testing, but only with appropriate specimen collection, namely, combined vaginal-rectal swabbing. In the future, it will be necessary to routinely include universal screening for GBS colonization in pregnancy in the Slovenian antenatal care system, for which the The Health Insurance Institute of Slovenia (ZZZS) funds will have to be provided. It will also be necessary to monitor the correct and efficient implementation of the programme and continue the search for new improvements.

Conflict of interest

None declared.

References

1. Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, et al. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet. 2015;385(9966):430-40. DOI: 10.1016/S0140-6736(14)61698-6 PMID: 25280870
2. Lučovnik M. Je napočil čas a uvedbo presejanja za kolonizacijo s strepotkokom skupine B v slovensko predporodno varstvo? In: Trojner Bregar A, Lučovnik M. 18. Novakovi dnevi. Zbornik prispevkov. Maj 2017; Slovenj Gradec, Slovenija. V Ljubljani: Združenje za perinatalno medicino SZD; 2017.
3. Edmond KM, Kortsalioudaki C, Scott S, Schrag SJ, Zaidi AK, Cousens S, et al. Group B streptococcal disease in infants aged younger than 3 months: systematic review and meta-analysis. Lancet. 2012;379(9815):547-56. DOI: 10.1016/S0140-6736(11)61651-6 PMID: 22226047
4. Lasič M, Lučovnik M, Pavčnik M, Kaparič T, Ciringer M, Krivec JL, et al. Invazivne okužbe novorojenčkov z bakterijo Streptococcus agalactiae v Sloveniji, 2003–2013. Zdrav Vestn. 2017;86(11-12):493-506.
5. Ouchenir L, Renaud C, Khan S, Bitnun A, Boisvert AA, McDonald J, et al. The epidemiology, management, and outcomes of bacterial meningitis in infants. Pediatrics. 2017;140(1):e20170476. DOI: 10.1542/peds.2017-0476 PMID: 28600447
6. Stoll BJ, Hansen NI, Sánchez PJ, Faix RG, Poindexter BB, Van Meurs KP, et al.; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Early onset neonatal sepsis: the burden of group B Streptococcal and E. coli disease continues. Pediatrics. 2011;127(5):817-26. DOI: 10.1542/peds.2010-2217 PMID: 21518717
7. Fišer J, Špacapan S, Prinčič D, Frelih T. Odkrivanje kolonizacije nosečnic z bakterijo Streptococcus agalactiae v severnoprimorski regiji. Zdrav Vestn. 2001;70:623-6.
8. Lučovnik M, Tul Mandić N, Lozar Krivec J, Kolenc U, Jeverica S. Prevalenca kolonizacije z bakterijo Streptococcus agalactiae pri nosečnicah v Sloveniji v obdobju 2013–2014. Zdrav Vestn. 2016;85(7-8):393-400.
9. Boyer KM, Gotoff SP. Prevention of early-onset neonatal group B Streptococcal disease with selective intrapartum chemoprophylaxis. NEJM. 1986;314(26):1665-9. DOI: 10.1056/NEJM198606263142603 PMID: 3520319
10. Fabjan Vodušek V, Cerar LK, Mole H, Šajina Stritar B, Pokorn M, Lučovnik M, et al. Antibiotično zdravljenje ob porodu – priporočila. Zdrav Vestn. 2019;88(1):93-102. DOI: 10.6016/ZdravVestn.2916
11. Van Dyke MK, Phares CR, Lynfield R, Thomas AR, Arnold KE, Craig AS, et al. Evaluation of universal antenatal screening for group B streptococcus. N Engl J Med. 2009;360(25):2626-36. DOI: 10.1056/NEJMoa0806820 PMID: 19535801
12. Verani JR, McGee L, Schrag SJ; Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention (CDC). Prevention of Perinatal Group B Streptococcal Disease: Revised Guidelines from CDC, 2010. MMWR Recomm Rep. 2010;59(RR10):1-32. PMID: 21088663
13. Bliss SJ, Manning SD, Tallman P, Baker CJ, Pearlman MD, Marrs CF, et al. Group B Streptococcus colonization in male and nonpregnant female university students: a cross-sectional prevalence study. Clin Infect Dis. 2002;34(2):184-90. DOI: 10.1086/338258 PMID: 11740706

14. Manning SD, Neighbors K, Tallman PA, Gillespie B, Marrs CF, Borchardt SM, et al. Prevalence of group B streptococcus colonization and potential for transmission by casual contact in healthy young men and women. Clin Infect Dis. 2004;39(3):380-8. DOI: 10.1086/422321 PMID: 15307006
15. Manning SD, Tallman P, Baker CJ, Gillespie B, Marrs CF, Foxman B. Determinants of co-colonization with group B streptococcus among heterosexual college couples. Epidemiology. 2002;13(5):533-9. DOI: 10.1097/00001648-200209000-00008 PMID: 12192222
16. Hansen SM, Uldbjerg N, Kilian M, Sørensen UB. Dynamics of Streptococcus agalactiae colonization in women during and after pregnancy and in their infants. J Clin Microbiol. 2004;42(1):83-9. DOI: 10.1128/JCM.42.1.83-89.2004 PMID: 14715736
17. Regan JA, Klebanoff MA, Nugent RP, Eschenbach DA, Blackwelder WC, Lou Y, et al.; VIP Study Group. Colonization with group B streptococci in pregnancy and adverse outcome. Am J Obstet Gynecol. 1996;174(4):1354-60. DOI: 10.1016/S0002-9378(96)70684-1 PMID: 8623869
18. Yancey MK, Schuchat A, Brown LK, Ventura VL, Markenson GR. The accuracy of late antenatal screening cultures in predicting genital group B streptococcal colonization at delivery. Obstet Gynecol. 1996;88(5):811-5. DOI: 10.1016/0029-7844(96)00320-1 PMID: 8885919
19. American College of Obstetricians and Gynecologists Committee on Obstetric Practice. Prevention of group B streptococcal early-onset disease in newborns: ACOG committee opinion, Number 797. Vol. 135. Obstet Gynecol. 2020;135:e51-72. DOI: 10.1097/AOG.0000000000003668 PMID: 31977795
20. Vovko P. Assesment of laboratory procedures for group B streptococci screening in pregnant women. Zdrav Vestn. 2007;76:33-9.
21. Six A, Firon A, Plainvert C, Caplain C, Bouaboud A, Touak G, et al. Molecular characterization of nonhemolytic and nonpigmented group B streptococci responsible for human invasive infections. J Clin Microbiol. 2016;54(1):75-82. DOI: 10.1128/JCM.02177-15 PMID: 26491182
22. Rosa-Fraile M, Spellerberg B. Reliable Detection of group B Streptococcus in the clinical laboratory. J Clin Microbiol. 2017;55(9):2590-8. DOI: 10.1128/JCM.00582-17 PMID: 28659318
23. Platt MW, McLaughlin JC, Gilson GJ, Wellhoner MF, Nims LJ. Increased recovery of group B Streptococcus by the inclusion of rectal culturing and enrichment. Diagn Microbiol Infect Dis. 1995;21(2):65-8. DOI: 10.1016/0732-8893(95)00022-3 PMID: 7628194
24. Badri MS, Zawaneh S, Cruz AC, Mantilla G, Baer H, Spellacy WN, et al. Rectal colonization with group B streptococcus: relation to vaginal colonization of pregnant women. J Infect Dis. 1977;135(2):308-12. DOI: 10.1093/infdis/135.2.308 PMID: 320278
25. Boyer KM, Gadzala CA, Kelly PD, Burd LI, Gotoff SP. Selective intrapartum chemoprophylaxis of neonatal group B streptococcal early-onset disease. II. Predictive value of prenatal cultures. J Infect Dis. 1983;148(5):802-9. DOI: 10.1093/infdis/148.5.802 PMID: 6355317
26. Buchan BW, Olson WJ, Mackey TL, Ledeboer NA. Clinical evaluation of the walk-away specimen processor and ESwab for recovery of Streptococcus agalactiae isolates in prenatal screening specimens. J Clin Microbiol. 2014;52(6):2166-8. DOI: 10.1128/JCM.00374-14 PMID: 24622104
27. Orenga S, James AL, Manafi M, Perry JD, Pincus DH. Enzymatic substrates in microbiology. J Microbiol Methods. 2009;79(2):139-55. DOI: 10.1016/j.mimet.2009.08.001 PMID: 19679151
28. Church DL, Baxter H, Lloyd T, Larios O, Gregson DB. Evaluation of StrepB select chromagar and the ast Track Diagnostics group B Streptococcus (GBS) real-time polymerase chain reaction assay compared to routine culture for detection of GBS during antepartum screening. J Clin Microbiol. 2017;55(7):2137-42. DOI: 10.1128/JCM.00043-17 PMID: 28446575
29. Craven RR, Weber CJ, Jennemann RA, Dunne WM. Evaluation of a chromogenic agar for detection of group B streptococcus in pregnant women. J Clin Microbiol. 2010;48(9):3370-1. DOI: 10.1128/JCM.00221-10 PMID: 20592154
30. Louie L, Kotowich L, Meaney H, Vearncombe M, Simor AE. Evaluation of a new chromogenic medium (StrepB select) for detection of group B Streptococcus from vaginal-rectal specimens. J Clin Microbiol. 2010;48(12):4602-3. DOI: 10.1128/JCM.01168-10 PMID: 20962144
31. Morita T, Feng D, Kamio Y, Kanno I, Somaya T, Imai K, et al. Evaluation of chromID strepto B as a screening media for Streptococcus agalactiae. BMC Infect Dis. 2014;14(1):46. DOI: 10.1186/1471-2334-14-46 PMID: 24479795
32. Poisson DM, Chandemerle M, Guinard J, Evrard ML, Naydenova D, Mesnard L. Evaluation of CHROMagar StrepB: a new chromogenic agar medium for aerobic detection of Group B Streptococci in perinatal samples. J Microbiol Methods. 2010;82(3):238-42. DOI: 10.1016/j.mimet.2010.06.008 PMID: 20600363
33. Salem N, Anderson JJ. Evaluation of four chromogenic media for the isolation of Group B Streptococcus from vaginal specimens in pregnant women. Pathology. 2015;47(6):580-2. DOI: 10.1097/PAT.0000000000000299 PMID: 26308132
34. Verhoeven PO, Noyel P, Bonneau J, Carricajo A, Fonsale N, Ros A, et al. Evaluation of the new brilliance GBS chromogenic medium for screening of Streptococcus agalactiae vaginal colonization in pregnant women. J Clin Microbiol. 2014;52(3):991-3. DOI: 10.1128/JCM.02926-13 PMID: 24403300
35. Block T, Munson E, Culver A, Vaughan K, Hryciuk JE. Comparison of carrot broth- and selective Todd-Hewitt broth-enhanced PCR protocols for real-time detection of Streptococcus agalactiae in prenatal vaginal/anorectal specimens. J Clin Microbiol. 2008;46(11):3615-20. DOI: 10.1128/JCM.01262-08 PMID: 18799703
36. El Aila NA, Tency I, Claeys G, Verstraelen H, Deschaght P, Decat E, et al. Comparison of culture with two different qPCR assays for detection of rectovaginal carriage of Streptococcus agalactiae (group B streptococci) in pregnant women. Res Microbiol. 2011;162(5):499-505. DOI: 10.1016/j.resmic.2011.04.001 PMID: 21514378
37. Couturier BA, Weight T, Elmer H, Schlaberg R. Antepartum screening for group B Streptococcus by three FDA-cleared molecular tests and effect of shortened enrichment culture on molecular detection rates. J Clin Microbiol. 2014;52(9):3429-32. DOI: 10.1128/JCM.01081-14 PMID: 25009049
38. Hernandez DR, Wolk DM, Walker KL, Young S, Dunn R, Dunbar SA, et al. Multicenter diagnostic accuracy evaluation of the Luminex Aries real-time PCR assay for group B Streptococcus detection in lim broth-enriched samples. J Clin Microbiol. 2018;56(8):1-9. DOI: 10.1128/JCM.01768-17 PMID: 29848562
39. Jeverica S, Kotnik E, Lučovnik M, Tul Mandić N. Ugotavljanje nosilstva bakterije Streptococcus agalactiae v nosečnosti – ali moramo v diagnostiki narediti naslednji korak? Zdrav Vestn. 2016;85:15-23.
40. Miller SA, Deak E, Humphries R. Comparison of the AmpliVue, BD MAX System, and Illumigene molecular assays for the detection of group B Streptococcus in antenatal screening specimens. J Clin Microbiol. 2015;53(6):1938-41. DOI: 10.1128/JCM.00261-15 PMID: 25788551
41. Shin JH, Pride DT. Comparison of three nucleic acid amplification tests and culture for detection of group B Streptococcus from enrichment broth. J Clin Microbiol. 2019;57(6):e01958-18. DOI: 10.1128/JCM.01958-18 PMID: 30944190
42. Di Renzo GC, Melin P, Berardi A, Blennow M, Carbonell-Estrany X, Donzelli GP, et al. Intrapartum GBS screening and antibiotic prophylaxis: a European consensus conference. J Matern Fetal Neonatal Med. 2015;28(7):766-82. DOI: 10.3109/14767058.2014.934804 PMID: 25162923
43. El Shahaway AA, El Maghraby HM, Mohammed HA, Abd Elhady RR, Abdelrhman AA. Diagnostic performance of direct latex agglutination, post-enrichment latex agglutination and culture methods in screening of group B streptococci in late pregnancy: a comparative study. Infect Drug Resist. 2019;12:2583-8. DOI: 10.2147/IDR.S203543 PMID: 31692504

44. Takayama Y, Matsui H, Adachi Y, Nihonyanagi S, Wada T, Mochizuki J, et al. Detection of Streptococcus agalactiae by immunochromatography with group B streptococcus-specific surface immunogenic protein in pregnant women. J Infect Chemother. 2017;23(10):678-82. DOI: 10.1016/j.jiac.2017.07.001 PMID: 28779876

45. Park CJ, Vandel NM, Ruprai DK, Martin EA, Gates KM, Coker D. Detection of group B streptococcal colonization in pregnant women using direct latex agglutination testing of selective broth. J Clin Microbiol. 2001;39(1):408-9. DOI: 10.1128/JCM.39.1.408-409.2001 PMID: 11191227

46. Subramaniam A, Blanchard CT, Ngek ES, Mbah R, Welty E, Welty T, et al.; Cameroon Health Initiative. Prevalence of group B streptococcus anogenital colonization and feasibility of an intrapartum screening and antibiotic prophylaxis protocol in Cameroon, Africa. Int J Gynaecol Obstet. 2019;146(2):238-43. DOI: 10.1002/ijgo.12870 PMID: 31127871

47. Furfaro LL, Chang BJ, Payne MS. Detection of group B Streptococcus during antenatal screening in Western Australia: a comparison of culture and molecular methods. J Appl Microbiol. 2019;127(2):598-604. DOI: 10.1111/jam.14331 PMID: 31120589

48. Silbert S, Rocchetti TT, Gostnell A, Kubasek C, Widen R. Detection of group B Streptococcus directly from collected ESwab samples by use of the BD Max GBS assay. J Clin Microbiol. 2016;54(6):1660-3. DOI: 10.1128/JCM.00445-16 PMID: 27053670

49. Virranniemi M, Raudaskoski T, Haapsamo M, Kauppila J, Renko M, Peltola J, et al. The effect of screening-to-labor interval on the sensitivity of late-pregnancy culture in the prediction of group B streptococcus colonization at labor: A prospective multicenter cohort study. Acta Obstet Gynecol Scand. 2019;98(4):494-9. DOI: 10.1111/aogs.13522 PMID: 30578547

50. Filkins L, Hauser JR, Robinson-Dunn B, Tibbetts R, Boyanton BL, Revell P. American Society for Microbiology provides 2020 guidelines for detection and identification of group B streptococcus. J Clin Microbiol. 2020;59(1):e19-2. DOI: 10.1128/JCM.01230-20 PMID: 33115849

51. Helmig RB, Gertsen JB. Diagnostic accuracy of polymerase chain reaction for intrapartum detection of group B streptococcus colonization. Acta Obstet Gynecol Scand. 2017;96(9):1070-4. DOI: 10.1111/aogs.13169 PMID: 28504863

52. Picchiassi E, Coata G, Babucci G, Giardina I, Summa V, Tarquini F, et al. Intrapartum test for detection of Group B Streptococcus colonization during labor. J Matern Fetal Neonatal Med. 2018;31(24):3293-300. DOI: 10.1080/14767058.2017.1369041 PMID: 28817995

53. Ramesh Babu S, McDermott R, Farooq I, Le Blanc D, Ferguson W, McCallion N, et al. Screening for group B Streptococcus (GBS) at labour onset using PCR: accuracy and potential impact - a pilot study. J Obstet Gynaecol. 2018;38(1):49-54. DOI: 10.1080/01443615.2017.1328490 PMID: 28764569

54. Furfaro LL, Chang BJ, Payne MS. Perinatal Streptococcus agalactiae epidemiology and surveillance targets. Clin Microbiol Rev. 2018;31(4):1-18. DOI: 10.1128/CMR.00049-18 PMID: 30111577

55. Shabayek S, Spellerberg B, Group B. Streptococcal colonization, molecular characteristics, and epidemiology. Front Microbiol. 2018;9:437. DOI: 10.3389/fmicb.2018.00437 PMID: 29593684

56. Da Cunha V, Davies MR, Douarre PE, Rosinski-Chupin I, Margarit I, Spinali S, et al.; DEVANI Consortium. Streptococcus agalactiae clones infecting humans were selected and fixed through the extensive use of tetracycline. Nat Commun. 2014;5(1):4544. DOI: 10.1038/ncomms5544 PMID: 25088811

57. Bekker V, Bijlsma MW, van de Beek D, Kuijpers TW, van der Ende A. Incidence of invasive group B streptococcal disease and pathogen genotype distribution in newborn babies in the Netherlands over 25 years: a nationwide surveillance study. Lancet Infect Dis. 2014;14(11):1083-9. DOI: 10.1016/S1473-3099(14)70919-3 PMID: 25444407

58. Perme T, Golparian D, Bombek Ihan M, Rojnik A, Lučovnik M, Kornhauser Cerar L, et al. Genomic and phenotypic characterisation of invasive neonatal and colonising group B Streptococcus isolates from Slovenia, 2001-2018. BMC Infect Dis. 2020;20(1):958. DOI: 10.1186/s12879-020-05599-y PMID: 33327946

59. Lamy MC, Dramsi S, Billoët A, Réglier-Poupet H, Tazi A, Raymond J, et al. Rapid detection of the "highly virulent" group B Streptococcus ST-17 clone. Microbes Infect. 2006;8(7):1714-22. DOI: 10.1016/j.micinf.2006.02.008 PMID: 16822689

60. Lartigue MF, Kostrzewa M, Salloum M, Haguenoer E, Héry-Arnaud G, Domelier AS, et al. Rapid detection of "highly virulent" Group B Streptococcus ST-17 and emerging ST-1 clones by MALDI-TOF mass spectrometry. J Microbiol Methods. 2011;86(2):262-5. DOI: 10.1016/j.mimet.2011.05.017 PMID: 21663770