Occurrence of virulence genes among *S. agalactiae* isolates from vagina and anus of pregnant women – a pilot study

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Abstract

Introduction: Streptococcus agalactiae, group B *Streptococcus* (GBS), is a leading cause of morbidity and mortality in neonates as well as in the elderly and in immunocompromised. The goal of this study was to evaluate the occurrence of chosen virulence genes, which are also responsible for the virulence of GBS, among strains isolated from women in the Department of Perinatology, Medical University of Lodz, Poland. *Material and methods:* Clinical swabs were collected at 35-37 weeks of pregnancy among 105 women. Forty GBS isolates were identified to the genus by using standard microbiological methods. Bacterial DNA was isolated with a Bacterial and Yeast Genomic DNA Kit and then PCR reactions were carried out to check the presence of virulence genes: *sag, scpB, bca* and *rib*. The standard bacterial strain, *Streptococcus agalactiae* ATCC 12386 was used as a control. Statistical analysis of relationship between the presence of particular virulence genes was carried out by using Chi^2 Pearson test. *Results*. Colonization with *S. agalactiae* was established in 31 of 105 women (29.5%) in this study. Vaginal colonization was shown in 9 women, rectal colonization in 13 women, colonization in both vagina and anus was found in 9 women. All the GBS strains (n = 40) had *sag* gene, 92.5% *scpB* gene. The presence of *rib* gene was confirmed in 14 of strains (35%), *bca* gene in 32 of strains (80%). Statistical analysis revealed no relationship between the presence of *bca* and *rib* genes. *Conclusions:* The frequency of occurrence of GBS strains among pregnant women is similar to other studies as well as the presence of *scpB* and *rib* genes. However, *bca* gene was found more often probably because of different population investigated.

Key words: *S. agalactiae*, virulence, *scpB, bca, rib*

Introduction

Streptococcus agalactiae, group B *Streptococcus* (GBS), is a leading cause of morbidity and mortality in neonates as well as increasing cause of infection in the elderly and in patients with chronic underlying medical conditions [1]. It belongs to the group of pyogenic streptococci, most species of this group are beta-hemolytic on blood agar plates and can cause a wide range of infectious diseases in human and animal hosts [2].

Maternal carriage has been recognized as the most important risk factor for GBS neonatal infection [3] and indeed, vertical transmission before or during delivery has been shown [4]. Mother-to-child transmission may lead to neonatal infection with mortality rates ranging from 10 to 20% [5]. However, the incidence of neonatal sepsis has fallen significantly because of widespread use of antibiotic prophylaxis for GBS carriers. Among pregnant women, the prevalence of colonization with GBS ranges from 3.2 to 36% [6, 7]. Unfortunately, prophylactic use of antibiotics is likely to contribute to the emergence of antibiotic-resistant GBS [8].

However, *S. agalactiae* strains are able to cause infections not only because the development of resistance but also due to their virulence traits. The most known factor responsible for virulence is capsule, but there are also others, such as surface protein Rib, alpha and beta antigens of the C protein or C5a peptidase. The last one is a surface enzyme, capable of inactivation of C5a component of human complement. It is encoded by *scpB* gene which may be transferred horizontally among pyogenic streptococci [9]. Additionally, ScpB facilitates binding of GBS strains to epithelial cells and extracellular matrix proteins (ECM) [10]. α-C protein participates in adherence, invasion of cervical epithelial cells, as well as resistance to phagocytosis [11, 12]. Most often it is expressed in Ia, Ib I and II *S. agalactiae* serotypes, rarely in III GBS serotype [13]. Rib protein has been found in

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significant percentage of GBS strains which caused invasive infections in neonates [14-16]. It was observed that the presence of antibodies against those proteins protected neonates from invasive infection by strains expressing Rib protein [17]. The main goal of this study was to evaluate the occurrence of chosen virulence genes among GBS strains isolated from pregnant women in the M. Madurowicz Regional Specialist Hospital in Lodz during 2010-2011.

Materials

The standard bacterial strain, *Streptococcus agalactiae* ATCC 12386, was used both as a control in phenotypic and in PCR reactions. Clinical *S. agalactiae* isolates were isolated from vagina (n = 18 isolates) and anus (n = 22) of 105 pregnant women at 35-37 weeks of pregnancy in the Department of Perinatology, Medical Unveristy of Lodz, Poland during 2010-2011. All study samples were collected by a gynecologist and transported to the Laboratory of Bacteriology within 4 hours on Amies transport media (Argenta). Standard and clinical strains were stored in –70°C for further investigation.

Methods

Forty GBS isolates were identified to the genus by using standard microbiological methods including: culture on the selective-differential medium for group B streptococci Strep B Select Agar (BioRad), on Agar medium with 5% of sheep blood (bioMerieux) to check hemolysis as well as by Pastorex Strep B latex test (BioRad). Speciation was confirmed by PCR for *Streptococcus agalactiae sag* gene [18]. Bacterial DNA was isolated with a Gene Matrix Bacterial and Yeast Genomic DNA Purification Kit (Blirt S.A.) according to the procedure. PCR reactions were carried out in a total volume of 25 µl in a Biometra cycler. The mixtures for *scpB*, *rib* and *bca* genes consisted of 0.5 U Hypernova DNA polymerase (DNA Gdańsk), 600 nM of each primer (IBB, Warsaw) [19-21], 2 mM $MgCl_2$ (DNA Gdańsk), 2.5 µl PCR buffer and 400 µM dNTP (Fermentas). The PCR profiles for *rib* and *bca* genes were as described previously [19, 20]; for *scpB* gene [21] PCR was performed as following: 35 cycles of 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min. The products were separated in 1-1.4% agarose gel (Prona) in 1 × TAE buffer (Fermentas) and stained with ethidium bromide (Sigma). Statistical analysis of relationship between the presence of particular virulence genes and site of isolation was carried out by using Chi^2 Pearson test.

Results

Colonization with *S. agalactiae* was established in 31 of 105 women (29.5%) in this study. Vaginal colonization was shown in 9 women, rectal colonization in 13 women and rectovaginal colonization in 9 women (n = 18 isolates). All the GBS strains phenotypically identified as *S. agalactiae* strains possessed *sag* gene. Only in 3 isolates *scpB* gene was not found (7.5%). The presence of *rib* gene was confirmed in 14 of strains (35%), *bca* gene in 32 of strains (80%). If the source of isolation is concerned, *rib* gene was found in 8 strains from vagina and 6 strains from anus. *bca* gene was found in 15 strains from vagina and 17 strains from anus (Table 1). Both genes were present in 5 strains from vagina, and 5 also from rectal swabs. There were several cases (n = 9 women; 22.5%) in which GBS strains were isolated both from vagina and anus, but in some of them (n = 3; 7.5%) the difference in the presence of virulence genes may suggest that there were different strains in both sources. However, those differences should be further confirmed by relatedness analysis. Statistical analysis revealed no significant relationship between the site of isolation of *S. agalactiae*, as well as no correlation between the presence of *bca* and *rib* genes.

Table 1. The presence of genes in *S. agalactiae* strains

Materials	*sag*		*scpB*		*bca*		*rib*	
	n	%	n	%	n	%	n	%
Swabs from vagina (n = 18)	18	100	16	88.9	15	83.3	8	44.4
Swabs from anus (n = 22)	22	100	21	95.45	17	77.3	6	27.3

Discussion

Colonization with *S. agalactiae* of 29.5% of women, as established in this study, is similar to results from other studies that report colonization rates between 10% and 36% [6, 7]. There are also other data showing surprisingly low colonization rates [22] – e.g. among Argentinian pregnant women it was only 3.2%. Similarly, in Poland data concerning colonization rates are diverse. Rectovaginal colonization with group B streptococci in latest years in Poland ranges from 20-30% [23-25]. The differences in this frequency may be explained by increasing carriage of GBS strains among pregnant women

as well as by some advances in diagnostic procedures [26].

Brimil *et al.* [27] showed that for a total of 34 GBS positive pregnant women, 32% carried GBS only vaginal, 24% only rectal and 44% both rectal and vaginal. According to Aila et al., it was respectively 24%, 22% and 53% [7]. In our studies 29.03% of women having GBS strains were carriers of *S. agalactiae* strains in vagina or both in vagina and rectum, and 41.93% had it in rectum. Therefore this data are similar to this obtained in previous studies but diverse when comparing to results obtained by Brzychczy-Włoch et al. which showed 80% colonization rate both in vagina and rectum, 11% only in rectum and 9% of women had GBS strains only in vagina. The discrepancies may result from diagnostic methods used or just from population diversity. However they emphasize the necessity of taking two swabs from pregnant women, both from vagina and anus, in order to avoid false negative results.

We have investigated the presence of some virulence traits of *S. agalactiae*, because they are also, beside of resistance to antibiotics, responsible for pathogenicity of that kind of bacteria. In our study we tested the presence of three virulence genes among strains isolated from pregnant women at 35-37 weeks of pregnancy in the M. Madurowicz Regional Specialist Hospital in Lodz during 2010-2011.

First gene, *scpB,* encodes streptococcal C5a peptidase, surface bacterial serine proteinase, and is located on a composite transposon [9]. ScpB shows the ability of inactivating of C5a of human complement. Additionally ScpB mediates GBS binding to human immobilized fibronectin, a large dimeric glycoprotein present in the extracellular matrix in a fibrillar form [10], and also plays a role in the invasion of GBS into epithelial cells [28]. That is why, that protein is thought to be a candidate for a vaccine, especially because of its conservative sequence, strong expression and extracellular localization [28, 29]. It was shown that using vaccines consisting of ScpB or ScpB–CPS conjugates facilitated eradication of *S. agalactiae* strains from lungs [28]. Some authors suggest that the presence of the *scpB* gene in human isolates is mandatory [9, 21], others [30] detected *scpB* in 97% of human isolates [31], but generally it is thought that only strains possessing *scpB* gene are infective for humans. According to Safadi et al. only two strains of 111 did not have *scpB* gene [32] and that reinforce the hypothesis that scpB-lmb region may be essential for the colonization or infection in humans [33, 34]. What is more, that gene encoding GBS surface protein constitute useful virulence factor to explain the ability of GBS to invade the central nervous system of neonates [32].

Very high prevalence of *scpB* gene among GBS human isolates justifies its use in PCR as a standard gene for checking GBS strains carriage. The data showed the highest efficacy of detecting GBS carriage while comparing the standard culture method, antigen detection, and *scpB* PCR (37%) [35]. Obviously, the possibility of detection of nonviable GBS cells by the antigen and PCR assays cannot be excluded, but several conditions could also explain the false-negative culture results (the impact of antibiotics, feminine hygiene products) [36]. In our study only three isolates described phenotypically as *S. agalactiae* were negative for *scpB* gene in PCR. This result is comparable with previous studies [32, 35].

An important virulence factor of GBS strains is also α-C surface protein (ACP), encoded by *bca* gene. It participates in invasion to cervical epithelial cells [12] and its activity is associated with the number of tandem repeats of 82-aminoacid fragment of this protein [37]. According to many research [14, 20, 38, 39] *bca* gene was generally found in Ia, Ib and II GBS serotypes, and in 15% of isolates of serotype V [14, 38].

The repeat numbers of the alpha C protein are not different between invasive and carriage strains [40], but variations in repeat number can alter their antigenicities and protective epitopes [41]. The variation of repeat numbers in *rib* was age related. Strains with few repeats were more often collected from neonates, and variants with larger numbers of repeats were more often collected from adults. This variation in repeat numbers can be attributed to the complex interaction of selective forces like bacterial virulence and counterforces like host immunity [42].

It was shown that between 30-55% of serotype Ia GBS human strains carry *bca* gene [19, 40]. *bac* was found more frequently among isolates from CSF than blood in invasive serotypes. However, differences in the frequency of specific genes were detected but it is possible that the encoded proteins were differentially expressed [43] and thus, differences in pathogenicity could be attributable to differences in gene expression [44]. In our study *bac* gene was detected in 32 of *S. agalactiae* strains (80%). It is generally high result when comparing to previous studies, where the occurrence ranged from 7.5-55% [19, 31, 40, 45]. However, there is also data showing the presence of *bca* gene in 100% of tested strains (type Ia strains) [43], but there only one serotype was tested.

Next investigated protein, surface protein Rib made of repeated sequences [46], is found in significant number of GBS strains isolated from invasive infections of neonates [14-16]. The presence of this protein, encoded by *rib* gene, is used as a marker in serotyping [17, 47]. It was shown that immunization of mice with components of purified Rib protein protected them from fatal infection caused by a strain possessing similar type of Rib protein [17]. A prior studies demonstrated that protein Rib [14] was present significantly more often in invasive versus colonizing serotype III strains, but *Mannig* et al. reported that the difference was marginal significant [43]. However, the possibility that the differences in collection dates and geographic location are responsible for this result cannot be excluded. The frequency of *rib* gene among macrolide-resistant isolates investigated in Poland amounted to 11% [44]. Our result is higher, *rib* gene was found in 14 of strains (35%), but study was not limited to resistant isolates.

There are also research that show some associations between the presence of virulence traits and the age of infected individuals. According to Ho et al. most *bca* genes (85.4%) were harbored in GBS strains from adult patients, while 59.2% of the *rib* genes were in GBS strains from neonates [42]. The associations were statistically significant between serotype III GBS with *rib* gene and neonatal early-onset infections and late-onset infections as well as between GBS infections of adults aged 16 to 50 years and GBS strains type Ib/*bca*.

Testing the relationship between the presence of *bca, scpB* and *rib* genes of *S. agalactiae* strains revealed that they were not associated (χ^2 test). Contrary to our results Dore et al. found some unadjusted pairwise associations between the *bca* and *rib* genes. According to that study, almost 50% of serotype III strains possessed the *bac* or *bca* genes or both, while 55-65% of the strains of serotypes Ia, Ib and II possessed the *rib* gene. These observations may indicate the presence of silent *bca* and *rib* genes in these serotypes [40]. Unfortunately, we also could not find any associations between the source of bacterial strains and the presence of particular genes ($p > 0.5$).

It has been hypothesized previously that the diffusion of strains of particular surface protein profiles and serotypes reflects the selection of the best evolutionary lineages by the immune system [48]. Our study indicate the presence of strains possessing some interesting features, e.g. high frequency of isolates having *bca* gene. Therefore some further investigations involving serotyping and molecular analysis of relatedness are needed to explain this result. Moreover, the limitation of our study may be small number of investigated strains, but we aim to conduct all the necessary research to obtain more precise data.

The prevalence of virulence genes among strains included in our study is high. However, the isolates assessed in it may contain other unknown virulence traits, as the virulence of GBS is probably attributable to multiple genes. It is also possible that these virulence genes may be differently expressed (or might have different impacts in other susceptible populations, eg. the elderly or those with underlying chronic disease). That is why there is need to conduct further research on the prevalence on virulence genes among pregnant women as well as check the serotype diversity of those GBS strains to get possibly complete data about pathogenicity of those bacteria.

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