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MOLECULAR EPIDEMIOLOGY OF *STREPTOCOCCUS AGALACTIAE* (GROUP B *STREPTOCOCCUS*)

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1. ABSTRACT

Group B *Streptococcus* (GBS) is a common cause of sepsis and meningitis in newborns, and causes disease in pregnant women and non-pregnant adults. The incidence of disease among non-pregnant adults, particularly those with underlying conditions, is increasing. In addition, many individuals are asymptomatically colonized with GBS. When compared to group A *Streptococcus* and *Streptococcus pneumoniae*, however, little is known about the pathogenesis, natural history and transmission dynamics of GBS. Various molecular tools have been utilized to study this organism, including both phenotypic techniques, such as serotyping and multilocus enzyme electrophoresis, and genotypic techniques such as plasmid analysis and pulsed-field gel electrophoresis. This review outlines the contributions of these methods to our current understanding of GBS infections.

2. INTRODUCTION

In recent years, the application of molecular tools to epidemiologic problems, or 'molecular epidemiology', has become increasingly more popular and important for health care providers, scientists, public health officials, and researchers in many different fields. Molecular epidemiology can be used to examine disease patterns, investigate outbreaks, describe transmission and population dynamics, understand evolution and disease pathogenesis, and identify uncultivable organisms (1, 2).

Little is known about the epidemiology, transmission and pathogenesis of Group B *Streptococcus* (GBS), or *Streptococcus agalactiae* when compared to other members of the *Streptococcus* family including *Streptococcus pneumoniae* and group A *Streptococcus*. This review will discuss the epidemiology of GBS; outline

specific GBS characteristics; examine various molecular methods used to study GBS and discuss the knowledge gained from them; highlight limitations associated with these methods; and emphasize specific GBS research areas that require further study.

3. EPIDEMIOLOGY OF GROUP B STREPTOCOCCUS

3.1. Disease presentation and incidence

GBS, a facultative gram-positive diplococcus with an ultrastructure similar to other gram-positive cocci (3, 4), was originally known for causing bovine mastitis (5-7) and was not demonstrated to be a human pathogen until 1938 (8). Currently, GBS remains a common cause of sepsis and meningitis in newborns despite prevention efforts (9), and can cause urosepsis, pneumonia, and skin and soft tissue infections, among non-pregnant adults with underlying medical conditions (10). During pregnancy, the primary clinical manifestation is bacteremia, but chorioamnionitis, endometritis and septic abortion also occur (11).

The incidence of early-onset newborn disease attributable to GBS has decreased from 1.7 to 0.6 cases per 1000 live births between 1993 and 1998, while the incidence among pregnant girls and women decreased from 0.29 in 1993 to 0.23 cases per 1000 deliveries in 1998 (11). In contrast, the number of GBS-attributable conditions among non-pregnant adults, particularly the elderly with underlying conditions, is increasing (10), ranging from 4.1 to 7.2 cases per 100,000 adults over the past decade (12-15).

3.2. Asymptomatic colonization

Many individuals are asymptomatically colonized with GBS. Depending on the characteristics of the study population and the detection methods utilized, 10 to 40% of individuals are colonized (16-18). Newborns over 48 hours old are most commonly colonized in the throat and rectum (19) and can remain colonized throughout childhood; little is known, however, about the duration of colonization. At the onset of sexual activity, colonization generally shifts to the genitourinary tract (5, 20-31).

3.3. Transmission and pathogenesis

Among newborns presenting with early-onset disease, GBS is generally acquired from the mother during labor and delivery (32), yet transmission can occur nosocomially (33-39) or via direct contact from other individuals (34, 35). Newborns with early-onset invasive disease have an increased density of epithelial cell surface receptors specific for GBS, (40) and some GBS strains can replicate more readily in amniotic fluid (41); both facilitate colonization, the first step in disease pathogenesis. Among adults, transmission is hypothesized to occur by the fecal-oral route or by person-to-person direct contact (30, 42). Few studies, however, have been conducted to directly assess this. The primary site of asymptomatic GBS colonization is the rectum, or the gastrointestinal tract (43, 44), with secondary spread to the genitourinary tract occurring frequently (16, 33).

The ability of certain GBS strains to move from a colonizing state to a disease-causing state is unclear, but many risk factors have been identified that may affect this process. Host factors, such as low type-specific maternal antibody levels at delivery (45-49), genetic predisposition (50-55), a compromised immune system or faulty immune response (20, 56), race/ethnicity (19, 31, 57, 58), and specific behavioral factors (e.g. sexual (30, 31, 57, 59)) in conjunction with different bacterial characteristics, such as virulence capacity (e.g., polysaccharide type (60)), adherence capabilities (61, 62, 63-71), host evasion mechanisms (72, 73), and inoculum density (32, 74) contribute to GBS colonization and subsequent disease. Like most infectious diseases, GBS disease pathogenesis is a multifactorial process in which colonization leads to invasion and clinical manifestations in certain individuals.

3.4. Risk factors

Maternal risk factors associated with the development of neonatal GBS disease, such as GBS colonization, premature delivery, prolonged rupture of membranes, intrapartum fever, GBS urinary tract infection, age less than 20 years, multiple pregnancies, history of GBS infection in a previous child, and African American ethnicity, have been identified (16, 19, 43, 57, 58, 74-76). Low birth weight, African American ethnicity, prematurity, low Apgar scores, and decreased level of maternal antibodies are examples of neonatal factors found to be associated with GBS disease (43, 75, 77). Among non-pregnant adults, most invasive disease occurs in the setting of underlying conditions including diabetes mellitus, cirrhosis, arteriosclerosis, malignancy or advanced age (10, 13, 32, 78). Further, those adults residing in a nursing home facility are 4 times more likely to have GBS disease when compared to age-matched individuals living in the community (79).

Only a few studies have been conducted on healthy, non-pregnant GBS carriers to identify risk factors associated with colonization. Ever engaging in sexual activity appears to be a common behavior associated with GBS colonization (25, 29, 31), as well as age less than 20 years, presence of an intrauterine device, time since last menses (25), tampon use, milk consumption, infrequent handwashing (29), use of yeast medication, and African American ethnicity (31). Sexual behaviors associated with colonization include: increased frequency of sexual intercourse, fewer days since last sex, acquiring a new sex partner, and having multiple sex partners (31). In a recent study conducted on heterosexual college couples, male-to-female oral sex, having four or more lifetime sex partners, and first sex at age 20 years or older were associated with co-colonization, or carriage of the genetically identical GBS strain among sex partners (30). Among pregnant women, diabetes mellitus (56, 80), age younger than 20 years (81, 82), and having fewer than three pregnancies (57, 81, 82) are associated with higher colonization rates. Others include: multiple sex partners, increased frequency of sexual activity, concurrent colonization with *Candida* sp., older age with lower parity, less education, and cohabitation with sex partners (57).

4. PREVENTION EFFORTS

In order to decrease the morbidity and mortality associated with GBS disease in newborns, the Centers for Disease Control and Prevention (CDC) and other organizations developed guidelines to provide women at risk of delivering a GBS-diseased infant with intrapartum prophylaxis (9, 16, 83, 84). As a result, at least 24% of women receive antibiotics during labor and delivery (9). Penicillin or ampicillin are the primary agents used to treat GBS infections. Clindamycin and erythromycin were recommended for use among penicillin-allergic women (16) until recently; resistance to both agents is increasing (85-90). Since the development of the GBS prevention program, the incidence of GBS neonatal sepsis has decreased significantly (11, 91), but the incidence of sepsis caused by *Escherichia coli* among very-low-birth-weight infants has increased by four percent. In addition, the majority of these *E. coli* were resistant to ampicillin (91).

Because antibiotic resistance is a major public health concern, alternative prevention protocols must be evaluated and implemented. For GBS, a good alternative to intrapartum prophylaxis is a vaccine, (92, 93) which should significantly reduce the number of women receiving antibiotics. This vaccine is under development, but is not available for use yet. In the interim, the threat of GBS acquiring resistance to penicillin is real and, therefore, it is imperative to gain a better understanding of the pathogenesis of GBS infections and identify host and bacterial characteristics important for disease progression.

5. OVERVIEW OF MOLECULAR METHODS

In the past, molecular epidemiologic approaches have been less critical for GBS when compared to other organisms since the source of adult and neonatal infection was generally known (32). Although this is true, recent studies conducted on GBS demonstrate that more molecular epidemiologic information will be useful to better understand the natural history of GBS and design alternative prevention strategies. For instance, comparing strains that cause disease with those that do not is important to identify other bacterial factors that may be important in disease pathogenesis. Also, examining strains that are shared among sex partners may reveal information regarding the transmission mode of GBS among nonpregnant adults.

Traditionally, phenotypic molecular techniques, such as serotyping, have been used to study GBS. Differentiating GBS strains by serotype enabled the earliest molecular epidemiologic studies to be conducted. More recently, genotypic techniques, such as pulsed-field gel electrophoresis and nucleotide sequence analyses, have been utilized to better characterize the GBS strains in circulation, and to gain a better understanding of disease pathogenesis, strain-to-strain variation, and possible transmission modes.

6. PHENOTYPIC CHARACTERISTICS

For GBS, serotyping is the most commonly used phenotypic assessment. Other systems that examine the bacterial proteins produced by GBS, such as the polysaccharide capsule, and evaluate their effect on the host, such as the immunologic response, also have been utilized. These phenotypic methods have contributed to our understanding of GBS infections, but they are limited since they do not reveal information regarding genetic identity, and they are often unable to differentiate between a large number of strains (94). Because of this, many investigators now use genotypic techniques alone or in conjunction with certain phenotypic techniques.

6.1. Serotype

The discovery of a group B-specific “C” substance and type-specific “S” substance led to the identification of four serotypes (Ia, Ib, II and III) (20, 95-101). Differences in the polysaccharide capsule are the basis for serotyping and since 1979, five more serotypes (IV-VIII) have been identified (102-108). To date, it has proven to be an extremely useful tool to study the epidemiology of GBS infections.

In the 1970’s and 1980’s GBS serotypes I, II and III predominated among asymptomatic newborns, newborns with early-onset disease, children, and adults (109-115). This serotype distribution has changed over time (19, 103, 116, 117). Serotype III still causes most cases of neonatal meningitis (20), yet serotype V has emerged to cause approximately 55% of all cases in the United States (14) with 10% to 15% targeting neonates (117-119). Serotypes VI-VIII are not frequently found in the United States and rarely contribute to disease (20) though the prevalence of serotype VIII is high in Japan (120, 121).

A similar distribution of serotypes has been observed among healthy, asymptomatic individuals: types Ia, III and V predominate in the United States (19). In a study of a random sample of male and female college students in Michigan conducted in 1998, serotype V (29%) and serotype III (20%) predominated (29).

In addition to determining which serotypes are responsible for disease, several researchers compared serotypes, as well as biochemical and biologic characteristics of GBS strains causing perinatal disease with strains causing bovine mastitis to determine if zoonotic transmission occurs (20, 122-126). In general, the virulence factors associated with bovine mastitis differ from those associated with human GBS strains (127) and there is no additional evidence supporting GBS transmission from cows to humans (20).

Serotyping does, however, have limitations in that it groups isolates into a few large categories and, when used alone, it may not be sensitive enough to detect important differences among epidemiologically unrelated strains (128). In fact, GBS serotypes are not homogenous groupings (14, 129-132) as considerable genetic variation

within each type has been found when assessed by various techniques, (118, 129, 131-134) with the exception of serotype V (14, 135, 136).

Another limitation associated with serotyping is that it requires specific reagents that may not be readily available and are too expensive for routine use in many research and clinical laboratories (137, 138). As discussed by Chaffin *et al*, developing a PCR-based serotyping system using specific primers that distinguish among the capsular types may alleviate this problem (139). Despite these limitations, serotyping is informative to use in conjunction with other molecular methods as it creates a means to compare strain distributions among distinct populations.

6.2. Antibiotic susceptibility profiles

Antibiograms have been used to characterize epidemiologically related GBS strains in several earlier studies. For example, Band *et al* used antibiotic susceptibility testing in conjunction with bacteriophage typing to determine that GBS strains isolated from three infants were related and acquired nosocomially (36). Antibiograms, however, are not useful for most epidemiologic studies since the expression of resistance can vary in one strain at different points in time. Because plasmids and mobile genetic elements often carry resistance genes, acquisition or loss of these elements can rapidly change the phenotype of any strain (140). Therefore, genetically different strains can have the same resistance patterns and visa versa. Furthermore, the development of resistance in many organisms is attributable to selective pressures, which usually varies depending on an individual's previous exposure to antibiotics; previous history of antibiotic use is the primary factor influencing resistance in many organisms (141). Because of this, some epidemiologically related strains might have different antibiograms.

6.3. Expressed virulence factors

Evaluating the expression of specific virulence factors *in vitro* or in animal models has been performed by many GBS investigators and has contributed greatly to our understanding of disease pathogenesis. The main problem with these studies, however, is that some virulence factors are only expressed in the host and not in the laboratory, thus the expression levels measured may not accurately reflect *in vivo* levels. This variable expression often decreases the reproducibility of the results (142).

6.3.1. Polysaccharide capsule

Similar to *S. pneumoniae*, the polysaccharide capsule, which is the basis for serotyping, is the major known virulence factor among GBS (41, 143, 144). Serotype III strains with increased capsule production are more resistant to opsonophagocytic killing (72, 73) by interfering with host complement factor C3b (72), and are more virulent (145). Therefore, a multivalent GBS vaccine targeting the more virulent GBS serotypes in circulation is under development (93). In general, many technical challenges must be overcome in order to make an effective capsule-containing vaccine, such as the inclusion of multiple capsular types, the identification and validation of a protein conjugate that enhances the immunogenicity, and the assessment of any cross-reactivity with human tissues (146). Those investigators working on the multivalent GBS vaccine, however, have made significant progress (93).

6.3.2. Alpha and beta C proteins

The cell surface antigens, particularly the C protein components, have been studied extensively. The C protein is common among many GBS strains and can contain a trypsin-sensitive (beta antigen) or trypsin-resistant (alpha antigen) component (147, 148). Both are independently expressed on the bacterial cell surface (149, 150). The C proteins are analogous to the variety of M-protein types found in group A streptococci (151), which are thought to play a role in virulence and immunity (127). Furthermore, organisms with C proteins have enhanced *in vitro* resistance to opsonophagocytosis (152, 153) and killing (154).

The alpha antigen is present in 50% (150) to 90% (73) of human GBS strains, yet there is considerable variation in the quantity of the alpha antigen produced by each strain (73), which may be related to virulence. In contrast, the beta antigen has been identified in only about 8% (19) to 42% (73) of human GBS strains. Several studies have demonstrated that the beta antigen acts as a receptor that binds human IgA via the Fc portion (155-160), thereby suggesting that it may inactivate the host immune response. This is functionally analogous to the Arp protein receptor found among group A streptococci (159), yet these two receptors show no homology at the protein level.

Several studies have demonstrated that antibodies directed against both the alpha and beta antigens offer protection from infection (73, 101, 148, 149, 161-165). Therefore, both may be useful vaccine candidates (164-166). In fact, it was estimated that a vaccine containing an alpha antigen and serotype III component would protect against at least 90% of GBS infections (167).

6.3.3. Other cell surface proteins

Some GBS strains also contain X and R antigens, which are immunologically cross-reactive cell surface proteins (4, 124, 168). Of the streptococcal R-proteins, R4 appears to be the most prevalent (169). Another surface protein designated Rib, confers protective immunity in mice (165, 170) and offers cross-protection in group A *Streptococcus* (171). It is also found in most serotype III strains (170), which are commonly associated with disease (20).

Two alpha-like proteins (172, 173) and a Rib-like protein (174) have been described, which suggests the existence of a family of GBS cell surface antigens (127, 172). Additionally, a cell surface protein designated Fbs (174) and a R-like protein named BPS (175) were identified as well as a glutamine synthetase (176), α-enolase (177), Hsp70 (178), and surface immunogenic protein (Sip) (179).

Unfortunately, the roles most of these proteins play in the disease process are unclear. For instance, two research teams observed no differential expression of alpha and beta proteins between pathogenic and colonizing GBS strains (180, 181). As with all assays that measure protein expression, however, it is possible that *in vitro* expression is altered and thus, the expression levels detected may not reflect the true levels *in vivo.* Furthermore, some researchers have reported that the GBS capsule may undergo phase variation (182-184) that may allow the organism to shift from a colonizing state to a disease-causing state (182). Because of the difficulties associated with expression assays, many investigators are now turning to genotypic assays that screen for the presence of genes that encode these proteins. This approach, however, also has limitations that will be discussed further.

6.4. Allelic variation at enzyme loci

It has been suggested that virulence determinants among many pathogenic species are not randomly distributed among different phylogenetic lineages (185). Therefore, several investigators have used multilocus enzyme electrophoresis (MLEE), which analyzes variations in protein mobility via starch gel electrophoresis to type GBS strains. The protein variations are due to charge differences that reflect amino acid substitutions in the chromosomal genes encoding them (138).

Musser *et al* demonstrated that serotype III GBS strains recovered from 18 states separated into two distantly related phylogenetic lines that differed in their ability to invade. They also identified a single serotype III clone that was associated with most invasive disease cases. Further characterization of isolates closely related to this highly virulent clone demonstrated that when compared to the less virulent strains they produced more extracellular hyaluronidase, an extracellular protein that cleaves hyaluronic acid present in the extracellular matrix of many tissues (133, 186). Other investigators, with the exception of Hauge *et al* (187), also have identified distinct groups of serotype III strains with enhanced virulence using this method (188-190).

These findings are important because they suggest that those GBS strains that cause disease may be unique and that identifying strains based on specific MLEE profiles might be an appropriate way to track virulence. Despite this, the few enzyme loci analyzed only represent a limited segment of the GBS genome, and thus, epidemiologically related and unrelated strains could have similar profiles. Additionally, MLEE is technically demanding and expensive and therefore, is difficult to perform outside of research and reference laboratories (191). In general, it should only be used to type GBS in conjunction with another molecular technique. Using MLEE alone, however, may be an appropriate tool to study phylogeny, or to define a group of strains that need further characterization.

7. GENOTYPIC CHARACTERISTICS

Genotypic techniques examine DNA differences between microorganisms of the same family, and are considered superior to phenotypic techniques in that they can more accurately identify epidemiologically related strains (128, 192). Further, phenotypic techniques often lack the typeability, reproducibility, and discriminatory power that genotypic methods have (138). For example, because many GBS strains within a serotype can be genetically similar (118, 131, 133, 189, 190) and most disease-causing microorganisms are relatively homogeneous when compared to other lineages (193), genotypic techniques are extremely helpful to identify subtle genetic differences between strains.

7.1. Plasmids

Decades ago, researchers recognized that the study of GBS was limited by the lack of a discriminatory molecular typing system. Plasmid analysis was the earliest molecular epidemiologic tool used to study genotypic characteristics of GBS to describe the relatedness between R plasmids isolated from groups B and D streptococci (194). Plasmids, however, are found infrequently among GBS (129), thereby limiting their usefulness as a typing system.

7.2. Bacteriophage

Bacteriophage typing was utilized in the early 1980's with serotyping data to study epidemiologically related GBS strains. The primary purpose was to determine whether several neonatal infections were acquired nosocomially or via the mother (37, 195), and to identify the source of infection in various nurseries (36, 196-198). Because bacteriophage typing requires the availability of specific reagents (130, 138), is labor-intensive (138), and has low discriminatory power when used alone (195), it is rarely used to study GBS.

7.3. Nucleotide sequences

Although sequencing the entire genome of various GBS disease-causing strains would be helpful for identifying important virulence gene combinations, this task has not been completed to date. In the interim, several investigators have focused their work on identifying and assessing the function of specific bacterial genes that may be important in disease pathogenesis. Describing the distribution of genes or gene sequences encoding putative virulence factors among a wide variety of disease-causing and colonizing isolates is critical to fully evaluate the role they play, and to assess their potential as vaccine candidates.

There are, however, limitations associated with screening strains for the presence or absence of nucleotide sequences. Primarily, strains containing the genes of interest may not be expressing the proteins and therefore, can be falsely classified. However, if the overall goal is to determine whether invasive strains are more likely to carry a specific gene when compared to colonizing strains, then screening for specific nucleotide sequences may be an easy first step. Genes found in fewer than 10% of invasive strains, for example, are likely not to be important in invasive disease, while those found in 60% of invasive strains may be important. Further studies, such as protein expression analyses, could then be conducted to better characterize each factor.

7.3.1. Putative virulence genes

Virulence factors other than capsule have been described and many are found in virtually all GBS isolates (*e.g*., hemolysin, CAMP, proteases, and LTA) (68, 117, 144, 199, 200). Although these most likely contribute to disease pathogenesis, they are generally expressed by both strains that cause disease and those that asymptomatically colonize the host. Consequently, the focus of this section will be on those factors with varying distributions in different human populations, at both the protein and gene level.

Because several studies have identified more virulent subgroups of GBS strains using different molecular typing methods (133, 189, 190), many investigators are concentrating on the identification of virulence factors that contribute to GBS pathogenicity. Currently, several putative virulence genes have been identified, cloned and sequenced. Three of these encode for the cell surface proteins alpha and beta antigen and Rib discussed earlier.

The gene encoding the alpha antigen, *bca*, was sequenced by Michel *et al* (162, 201), and is homologous to the antiphagocytic M-proteins of group A streptococci (201). The *bca* gene contains nine identical tandem repeats (201); the antigenicity (163, 202, 203) and pathogenicity (204) of strains containing the alpha antigen is dependant on the number of repeats expressed (73, 202). Furthermore, *bca* mutants have reduced virulence in neonatal mice (127).

The beta antigen is encoded by the *bac* gene (158, 159), which is present in 19% of strains from asymptomatically colonized newborns (205) to 46% of GBS strains from adult and newborn cases (206). Rib, encoded by the *rib* gene, also contains numerous repeats. It has 47% identity at the amino acid level to the alpha antigen repeat region, but has no immunologic cross-reactivity (207). Additionally, it is expressed in most serotype III strains (170), which are more commonly associated with invasive neonatal infections (20).

Although these reports strongly support a role for these antigens in disease pathogenesis, the gene distributions are confusing. For instance, no difference in the frequency of *bca* was identified between newborns with sepsis, and asymptomatic newborns and pregnant women from Germany (205). There is a possibility, however, that the proteins are differentially expressed as described previously (208).

Other putative virulence genes identified by a variety of methods encode a surface immunogenic protein (*sip*) (179), a placental laminin binding protein (*lmb*) (70), an R-like protein antigen (*alp4*) (209), a glutamine synthetase *(glnA*) (176), a protein important for cell division and antibiotic tolerance (*pcsB*) (210), a superoxide dismutase (*sodA*) (211), a glutamine transport gene (*glnQ*) (212), an adhesin and C5a protease (*scpB*) (71), a two-component signal transduction system (*rgfBDAC*) (213), and numerous outer surface proteins with homology to *Streptococcus pneumoniae* (214). Most of these have been discovered only recently and, thus, have not been studied enough to fully evaluate their role in disease pathogenesis.

7.3.2. Mobile genetic elements

Several interesting insertion elements that may be important for GBS disease have been identified and sequenced. Hyaluronidase, demonstrated to be an important virulence factor in various studies (133, 215), is thought to play a role in bacterial dissemination (216). Granlund *et al* identified an insertion element (IS*1548*) present in 9 of 13 isolates originating from the blood of endocarditis patients and in only 3 of 22 asymptomatically colonized women. The insertion was located within the hyaluronidase gene (*hylB*) in most cases and therefore, prevented expression. The authors speculate that IS*1548* may be associated with GBS strains that specifically cause endocarditis but not neonatal infections (186).

The insertion element ISSa*4*, which belongs to the IS*982* family, also was present in GBS, yet only in those strains isolated after 1996 suggesting it was recently acquired (217). The role of ISSa*4* in GBS disease has not been elucidated. Another insertion sequence IS*1381*, previously found in multiple copies in *Streptococcus pneumoniae* strains (218), also was identified in GBS. Screening for the presence of IS*1381* proved to be a useful subtyping tool to identify epidemiologically linked GBS isolates (219).

7.4. DNA fingerprinting

7.4.1. Restriction endonuclease analyses

Several techniques involving restriction endonucleases, enzymes that cleave DNA at specific nucleotide sequences, have been used for DNA fingerprinting to type GBS. Restriction endonuclease analysis (REA) of chromosomal DNA digested with restriction enzymes and resolved by linear gel electrophoresis has answered several important epidemiologic questions (129, 132, 134, 220). First, Denning *et al* used REA to determine that two GBS-associated relapses in an infant were due to the originally infecting GBS strain (221) suggesting that reinfection does occur. In another study by Denning *et al* on 54 human and animal GBS strains conducted to assess the geographic distribution of specific strains, 28 REA patterns from six different serotype groups and one nontypable group were identified. All epidemiologically related isolates matched by REA analysis, and genetically identical strains were recovered from different geographic locations suggesting that specific clones were in circulation (129). Additionally, Ellis and colleagues found that GBS strains isolated from mother/baby pairs had similar restriction patterns, which provided additional evidence for vertical transmission (134).

REA also has been used to study the population structure of GBS. Takahashi *et al* subclassified serotype III strains from Tokyo and Utah into three distinct phylogenetic lineages using REA with two different enzymes (*Hin*dIII and *Sse*83871). One lineage was composed of 91% of the invasive neonatal GBS isolates tested, which suggests that strains in this lineage may be

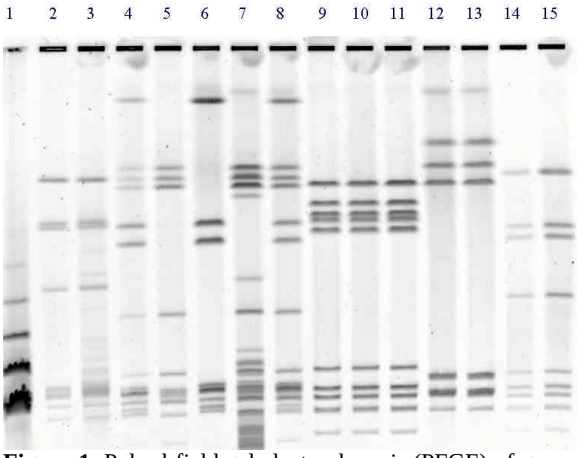

Figure 1. Pulsed-field gel electrophoresis (PFGE) of group B *Streptococcus* (GBS) strains isolated from college women with urinary tract infections and their most recent sex partner. Lane 1 is a DNA ladder. Lanes 2-3 and 14-15 represent rectal and vaginal GBS isolates from two different women. Lanes 4-6 are two urine isolates and one rectal isolate from one woman and lanes 7 and 8 are the urine and rectal isolate from her sex partner, respectively. Lanes 9-11 represent a female vaginal isolate and her sex partner's urine and rectal isolate.

more virulent (220). Further characterization of these strains using genomic subtractive hybridization revealed nine unique DNA sequences that were not found in other serotypes or type III lineages (222). Six of these nine sequences mapped to a fragment that contained or was located near known GBS virulence genes (223).

Although REA has enabled researchers to differentiate epidemiologically related strains and to identify a subset of more virulent strains, the complex banding patterns that result are often difficult to interpret. In order to avoid the problems associated with interpreting REA results, DNA or rRNA hybridization procedures have been used. This technique highlights specific fragments that vary in length between strains (restriction fragment length polymorphisms (RFLPs)). For GBS, this method was first used by Bingen and colleagues on total chromosomal DNA and ribosomal DNA (ribotyping) to determine whether GBS was transmitted to infants via maternal breast milk (130). When total GBS DNA was assessed, strains from mother-infant pairs produced 10 unique RFLP patterns, and those strains shared by the mother and infant within each pair were genetically identical. This is consistent with other findings among different mother-infant pairs and twin-twin strains (118). The ribotyping analysis was less discriminatory and yielded fewer patterns in this analysis (130) as well as another study (224); therefore, few investigators currently use it to characterize epidemiologically related GBS strains. Because ribosomal sequences are relatively stable within bacterial species, different strains can have identical ribotype patterns (138, 225, 226). Consequently, ribotyping is useful to study the phylogeny of GBS, but when used for typing, it should be performed in conjunction with another typing method to enhance the discriminatory power (224, 227-229).

7.4.2.Pulsed-field gel electrophoresis

Digestion of chromosomal DNA with a rare-cutting enzyme and pulsed-field gel electrophoresis (PFGE) also will produce fewer DNA fragments, thereby making interpretations easier (Figure 1). PFGE, which is considered the method of choice for outbreak investigations (230), is extremely reproducible, has high discriminatory power (231), and thus, appears to be a good typing technique for GBS (20, 232) and most other bacteria (191).

Green *et al* first used PFGE to demonstrate that recurring GBS disease in infants could be attributed to either the original infecting strain or a newly acquired strain (233). Ellis and colleagues identified a single clone associated with neonatal infections within serotype III strains from two geographically distinct populations in Australia. They concluded that a highly virulent clone may be widely distributed and more likely to contribute to neonatal disease (134). This is consistent with previous reports using alternative typing methods (133, 188-190, 220, 234, 235). In other studies, PFGE was used to demonstrate that individuals can be colonized with more than one genetically distinct strain at different sites and time points (29), and to illustrate that sex partners frequently share genetically identical GBS strains (30).

In addition to being expensive and laborious, the primary limitation associated with PFGE is that it may be too sensitive in some instances since minor genetic events are often reflected by the gain or loss of a restriction site (236). However, this does not apply to GBS. For example, most serotype V strains have identical banding patterns [unpublished data], which suggests the dissemination of a single clone. Utilizing more sensitive techniques to study these strains may reveal subtle genetic differences that cannot be detected by PFGE. This approach, however, may not be epidemiologically useful.

7.4.3.Polymerase chain reaction (PCR)-based methods

Random amplified polymorphic DNA (RAPD) analysis, a PCR-based typing method, uses short arbitrary nucleotide sequences as primers to discriminate between strains (237, 238). Chatellier *et al* examined a GBS collection that was previously characterized by MLEE and found that both methods were in agreement with regard to the groupings obtained, yet this occurred only when all four PCR primers were used together. Interestingly, RAPD subdivided the strains further than MLEE, and therefore, they concluded that RAPD was more sensitive to type GBS for epidemiologic investigations, particularly when combined with serotyping (235). This was demonstrated previously by Limansky *et al* (239) and for other pathogens as well (240). RAPD has also been utilized to type GBS strains with resistance to erythromycin. Culebras *et al* determined that resistance was not attributable to the dissemination of a single clone since 35 RAPD patterns were observed from the 54 resistant GBS strains studied (241).

Although RAPD represents a quick, easy and inexpensive way to group GBS isolates, there are also limitations associated with its use. PCR products are easily contaminated and it is often challenging to identify suitable primers that yield consistent results. Also, the technique is susceptible to variations in PCR conditions, and thus, the results can be difficult to reproduce (138, 231).

8. SUMMARY

Because GBS contributes to substantial morbidity and mortality in many individuals, it is surprising that our knowledge of the GBS genome and the genetic factors that enhance pathogenicity is so limited. Further, epidemiologic research is still lacking in key areas, such as understanding the GBS transmission modes and the duration of colonization.

Recent advances in molecular analyses, particularly the genotypic techniques, have been applied to GBS and have revealed important information regarding the pathogenesis of disease. For example, several studies have demonstrated that strains causing certain types of invasive disease represent a unique, but relatively homogeneous group (133, 188-190, 220, 234, 235), which is consistent with findings from other human pathogens (193). Because of these studies and others that have utilized molecular epidemiologic approaches, we now have a more detailed understanding of the genetic differences between disease-causing and colonizing strains. When compared to other microorganisms including *Streptococcus pneumoniae* and group A *Streptococcus*, however, our knowledge is still relatively limited.

9. FUTURE WORK

Since GBS has acquired resistance to several of the commonly used antibiotics, such as erythromycin and clindamycin, (10, 86) and the emergence of penicillin resistance is a possibility, prevention protocols other than intrapartum chemoprophylaxis must be utilized to prevent neonatal disease. Further, the incidence of adult disease is increasing (10), and currently, there is no established prevention protocol for this population. Therefore, future work should focus on gaining a better understanding of the genetic profiles of the more virulent GBS strains in circulation nationwide. This could facilitate the development of a screening program. For instance, Chatellier and colleagues suggest that RAPD-PCR could be used to identify GBS isolated from individuals that yield specific RAPD banding patterns indicative of enhanced virulence (235). These patterns could represent a marker for high disease risk and could aid in the implementation of prevention strategies. In general, establishing convenient phenotypic or genotypic criteria for strains with enhanced virulence would help health care providers identify individuals with a high risk of infection.

More work should also concentrate on identifying and analyzing novel bacterial characteristics that may be important in GBS disease via cloning, sequencing, mutagenesis, subtractive hybridization techniques, or microarray-based expression profiling analyses. Further epidemiologic studies should be conducted to assess whether those bacterial factors identified are more frequently found in disease-causing strains. If relevant, putative virulence factor components could be incorporated into the multivalent GBS vaccine that will be comprised of the most virulent polysaccharide capsular types (242). This would provide added protection in case the serotype distribution changes over the next few decades as was demonstrated previously (19, 103, 116, 117). If this vaccine was administered to pregnant women today, eradication of GBS disease, at least among neonates, pregnant women receiving the vaccine, and future elderly female populations could be realized given that the non-vaccine serotypes do not change in their ability to cause disease.

Because the GBS vaccine is currently not ready for clinical use, more knowledge on the natural history of GBS is critical. For instance, little is known about the GBS transmission dynamics in non-pregnant and pregnant adults. Preventing colonization, the initial step in disease pathogenesis, also could have an impact on the incidence of GBS disease in various populations, as well as gaining a better understanding of the complex interactions between the host and bacterium.

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11. REFERENCES

1. Foxman, B. & Riley, L.: Molecular epidemiology: Focus on infection. *Am J Epidemiol* 153, 1135-1141 (2001)

2. Skuce, R.A. & Neill, S.D.: Molecular epidemiology of *Mycobacterium bovis*: Exploiting molecular data. *Tuberculosis* 81, 169-175 (2001)

3. Kasper, D.L. & Baker, C.J.: Electron microscopic definition of surface antigens of group B *Streptococcus*. *J Infect Dis* 139, 147-151 (1979)

4. Wagner, B., Wagner, M., Kubin, V. & Ryc, M.: Immunoelectron microscopic study of the location of group-specific and protein type-specific antigens of group B streptococci. *J Gen Microbiol* 118, 95-105 (1980)

5. Anthony, B.F. & Okada, D.M.: The emergence of group B streptococci in infections of the newborn infant. *Annu Rev Med* 28, 355-369 (1977)

6. Brown, J.H.: Cultural differentiation of beta-hemolytic streptococci of human and bovine sources. *J Exp Med* 31, 35-45 (1920)

7. Stableforth, A.W.: Incidence of various serological types of *Streptococcus agalactiae* in herds of cows in Great Britain. *Journal Pathol Bacteriol* 46, 21-29 (1938)

8. Fry, R.M.: Fatal infections caused by haemolytic *Streptococcus* group B. *Lancet* 1, 199-201 (1938)

9. Schrag, S.J., Zell, E.R., Lynfield, R., Roome, A., Arnold, K.E., Craig, A.S., Harrison, L.H., Reingold, A., Stefonek, K., Smith, G., Gamble, M. & Schuchat, A.: A population-based comparison of strategies to prevent early-onset group B streptococcal disease in neonates. *N Engl J Med* 347, 233-239 (2002)

10. Farley, M.M.: Group B streptococcal disease in nonpregnant adults. *Clin Infect Dis* 33, 556-561 (2001)

11. Schrag, S.J., Zywicki, S., Farley, M.M., Reingold, A.L., Harrison, L.H., Lefkowitz, L.B., Hadler, J.L., Danila, R., Cieslak, P.R. & Schuchat, A.: Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. *N Engl J Med* 342, 15-20 (2000)

12. Schwartz, B., Schuchat, A., Oxtoby, M.J., Cochi, S.L., Hightower, A. & Broome, C.V.: Invasive group B streptococcal disease in adults. A population-based study in metropolitan Atlanta. *JAMA* 266, 1112-1114 (1991)

13. Farley, M.M., Harvey, R.C., Stull, T., Smith, J.D., Schuchat, A., Wenger, J.D. & Stephens, D.S.: A population-based assessment of invasive disease due to group B Streptococcus in nonpregnant adults. *N Engl J Med* 328, 1807-1811 (1993)

14. Blumberg, H.M., Stephens, D.S., Modansky, M., Erwin, M., Elliot, J., Facklam, R.R., Schuchat, A., Baughman, W. & Farley, M.M.: Invasive group B streptococcal disease: the emergence of serotype V. *J Infect Dis* 173, 365-373 (1996)

15. Munoz, P., Llancaqueo, A., Rodriguez-Creixems, M., Pelaez, T., Martin, L. & Bouza, E.: Group B streptococcus bacteremia in nonpregnant adults. *Arch Intern Med* 157, 213-216 (1997)

16. Centers for Disease Control and Prevention.: Prevention of perinatal group B streptococcal disease: a public health perspective. *MMWR* 45, 1-24 (1996)

17. Schuchat, A., Oxtoby, M., Cochi, S., Sikes, R.K., Hightower, A., Plikaytis, B. & Broome, C.V.: Population-based risk factors for neonatal group B streptococcal disease: results of a cohort study in metropolitan Atlanta. *J Infect Dis* 162, 672-677 (1990)

18. Dillon, H.C., Jr., Gray, E., Pass, M.A. & Gray, B.M.: Anorectal and vaginal carriage of group B streptococci during pregnancy. *J Infect Dis* 145, 794-799 (1982)

19. Hickman, M.E., Rench, M.A., Ferrieri, P. & Baker, C.J.: Changing epidemiology of group B streptococcal colonization. *Pediatr* 104, 203-209 (1999)

20. Baker, C.J. & Edwards, M.S.: Group B streptococcal infections. In: Infectious disease of the fetus and newborn infant. Eds: Remington, J.S. and Klein, J.O., WB Saunders, Philadelphia 1091-1156 (2001)

21. Baker, C.J. & Edwards, M.S.: Group B streptococcal infections. Perinatal impact and prevention methods. *Ann NY Acad Sci* 549, 193-202 (1988)

22. Hammerschlag, M.R., Baker, C.J., Alpert, S., Kasper, D.L., Rosner, I., Thurston, P., Webb, B.J. & McCormack, W.M.: Colonization with group B streptococci in girls under 16 years of age. *Pediatr* 60, 473-476 (1977)

23. Maurer, M., Thirumoorthi, M.C. & Dajani, A.S.: Group B streptococcal colonization in prepubertal children. *Pediatr* 64, 65-67 (1979)

24. Wallin, J. & Forsgren, A.: Group B streptococci in venereal disease clinic patients. *Br J Vener Dis* 51, 401-404 (1975)

25. Baker, C.J., Goroff, D.K., Alpert, S., Crockett, V.A., Zinner, S.H., Evrard, J.R., Rosner, B. & McCormack, W.M.: Vaginal colonization with group B *Streptococcus*: a study in college women. *J Infect Dis* 135, 392-397 (1977)

26. Gardner, S.E., Yow, M.D., Leeds, L.J., Thompson, P.K., Mason, E.O., Jr. & Clark, D.J.: Failure of penicillin to eradicate group B streptococcal colonization in the pregnant woman. A couple study. *Am J Obstet Gynecol* 135, 1062-1065 (1979)

27. Manuel, F.R., MacDonald, S.W. & Embil, J.A.: Prevalence of group B beta-hemolytic streptococci in the male urethra. *Scand J Infect Dis* 12, 33-35 (1980)

28. Lewis, R.F.: Beta-haemolytic streptococci from the female genital tract: clinical correlates and outcome of treatment. *Epidemiol Infect* 102, 391-400 (1989)

29. Bliss, S.J., Manning, S.D., Tallman, P., Baker, C.J., Pearlman, M.D., Marrs, C.F. & Foxman, B.: Group B *Streptococcus* colonization in male and non-pregnant female University students: A cross-sectional prevalence study. *Clin Infect Dis* 34, 184-190 (2002)

30. Manning, S.D., Tallman, P., Baker, C.J., Gillespie, B., Marrs, C.F. & Foxman, B.: Determinants of co-colonization with group B streptococcus among heterosexual college couples. *Epidemiology* 13, 533-539 (2002)

31. Meyn, L.A., Moore, D.M., Hillier, S.L. & Krohn, M.A.: Association of sexual activity with colonization and vaginal acquisition of group B *Streptococcus* in nonpregnant women. *Am J Epidemiol* 155, 949-957 (2002)

32. Schuchat, A.: Epidemiology of group B streptococcal disease in the United States: shifting paradigms. *Clin Microbiol Rev* 11, 497-513 (1998)

33. Aber, R.C., Allen, N., Howell, J.T., Wilkenson, H.W. & Facklam, R.R.: Nosocomial transmission of group B streptococci. *Pediatr* 58, 346-353 (1976)

34. Paredes, A., Wong, P., Mason, E.O., Jr., Taber, L.H. & Barrett, F.F.: Nosocomial transmission of group B

streptococci in a newborn nursery. *Pediatr* 59, 679-682 (1977)

35. Anthony, B.F., Okada, D.M. & Hobel, C.J.: Epidemiology of the group B *Streptococcus*: maternal and nosocomial sources for infant acquisitions. *J Pediatr* 95, 431-436 (1979)

36. Band, J.D., Clegg, H.W.D., Hayes, P.S., Facklam, R.R., Stringer, J. & Dixon, R.E.: Transmission of group B streptococci. Traced by use of multiple epidemiologic markers. *Am J Dis Child* 135, 355-358 (1981)

37. Easmon, C.S., Hastings, M.J., Clare, A.J., Bloxham, B., Marwood, R., Rivers, R.P. & Stringer, J.: Nosocomial transmission of group B streptococci. *Br Med J (Clin Res Ed)* 283, 459-461 (1981)

38. Easmon, C.S., Hastings, M.J., Blowers, A., Bloxham, B., Deeley, J., Marwood, R., Rivers, R.P. & Stringer, J.: Epidemiology of group B streptococci: one year's experience in an obstetric and special care baby unit. *Br J Obstet Gynaecol* 90, 241-246 (1983)

39. Gerards, L.J., Cats, B.P. & Hoogkamp-Korstanje, J.A.: Early neonatal group B streptococcal disease: degree of colonisation as an important determinant. *J Infect* 11, 119-124 (1985)

40. Broughton, R.A. & Baker, C.J.: Role of adherence in the pathogenesis of neonatal group B streptococcal infection. *Infect Immun* 39, 837-843 (1983)

41. Baker, C.J. & Edwards, M.S.: Group B streptococcal infections. In: Infectious disease of the fetus and newborn infant. Eds: Remington, J.S. and Klein, J.O., W.B. Saunders, Philadelphia 980-1054 (1995)

42. Hill, H.R.: Group B streptococcal infections. In: Sexually transmitted diseases, Second Edition. Eds: Holmes, K.K., Mardh, P.A., Sparling, P.F. and Wiesner, P.J., McGraw-Hill, New York, NY 851-861 (1990)

43. Baker, C.J. & Barrett, F.F.: Transmission of group B streptococci among parturient women and their neonates. *J Pediatr* 83, 919-925 (1973)

44. Badri, M.S., Zawaneh, S., Cruz, A.C., Mantilla, G., Baer, H., Spellacy, W.N. & Ayoub, E.M.: Rectal colonization with group B *Streptococcus*: relation to vaginal colonization of pregnant women. *J Infect Dis* 135, 308-312 (1977)

45. Baker, C.J. & Kasper, D.L.: Correlation of maternal antibody deficiency with susceptibility to neonatal group B streptococcal infection. *N Engl J Med* 294, 753-756 (1976)

46. Baker, C.J., Kasper, D.L., Tager, I., Paredes, A., Alpert, S., McCormack, W.M. & Goroff, D.: Quantitative determination of antibody to capsular polysaccharide in infection with type III strains of group B *Streptococcus*. *J Clin Invest* 59, 810-818 (1977)

47. Vogel, L.C., Boyer, K.M., Gadzala, C.A. & Gotoff, S.P.: Prevalence of type-specific group B streptococcal antibody in pregnant women. *J Pediatr* 96, 1047-1051 (1980)

48. Boyer, K.M., Papierniak, C.K., Gadzala, C.A., Parvin, J.D. & Gotoff, S.P.: Transplacental passage of IgG antibody to group B *Streptococcus* serotype Ia. *J Pediatr* 104, 618-620 (1984)

49. Flores, A.E., Nelson, J.A., Wu, X.Y. & Ferrieri, P.: Antibody profiles to the group B streptococcal beta antigen in maternal and infant paired sera. *APMIS* 101, 41-49 (1993)

50. Grubb, R., Christensen, K.K., Christensen, P. & Linden, V.: Association between maternal Gm allotype and neonatal septicaemia with group B streptococci. *J Immunogenet* 9, 143-147 (1982)

51. Christensen, K.K., Christensen, P., Hagerstrand, I., Linden, V., Nordbring, F. & Svenningsen, N.: The clinical significance of group B streptococci. *J Perinat Med* 10, 133-146 (1982)

52. Oxelius, V.A., Linden, V., Christensen, K.K. & Christensen, P.: Deficiency of IgG subclasses in mothers of infants with group B streptococcal septicemia. *Int Arch Allergy Appl Immunol* 72, 249-252 (1983)

53. Rundgren, A.K., Christensen, K.K. & Christensen, P.: Increased frequency of high serum IgM among mothers of infants with neonatal group-B streptococcal septicemia. *Int Arch Allergy Appl Immunol* 77, 372-373 (1985)

54. Christensen, K.K., Christensen, P., Faxelius, G., Henrichsen, J. & Pedersen, F.K.: Immune response to pneumococcal vaccine in mothers to infants with group B streptococcal septicemia: evidence for a divergent IgG/IgM ratio. *Int Arch Allergy Appl Immunol* 76, 369-372 (1985)

55. Thom, H., Lloyd, D.L. & Reid, T.M.: Maternal immunoglobulin allotype (Gm and Km) and neonatal group B streptococcal infection. *J Immunogenet* 13, 309-314 (1986)

56. Matorras, R., Garcia-Perea, A., Usandizaga, J.A. & Omenaca, F.: Recto-vaginal colonization and urinary tract infection by group B *Streptococcus* in the pregnant diabetic patient. *Acta Obstet Gynecol Scand* 67, 617-620 (1988)

57. Regan, J.A., Klebanoff, M.A. & Nugent, R.P.: The epidemiology of group B streptococcal colonization in pregnancy. Vaginal Infections and Prematurity Study Group. *Obstet Gynecol* 77, 604-610 (1991)

58. Campbell, J.R., Hillier, S.L., Krohn, M.A., Ferrieri, P., Zaleznik, D.F. & Baker, C.J.: Group B streptococcal colonization and serotype-specific immunity in pregnant women at delivery. *Obstet Gynecol* 96, 498-503 (2000)

59. Newton, E.R., Butler, M.C. & Shain, R.N.: Sexual behavior and vaginal colonization by group B

Streptococcus among minority women. *Obstet Gynecol* 88, 577-582 (1996)

60. Wessels, M.R., Rubens, C.E., Benedi, V.J. & Kasper, D.L.: Definition of a bacterial virulence factor: sialylation of the group B streptococcal capsule. *Proc Natl Acad Sci USA* 86, 8983-8987 (1989)

61. Mardh, P.A. & Westtom, L.: Adherence of bacterial to vaginal epithelial cells. *Infect Immun* 13, 661-666 (1976)

62. Ofek, I., Beachey, E.H., Eyal, F. & Morrison, J.C.: Postnatal development of binding of streptococci and lipoteichoic acid by oral mucosal cells of humans. *J Infect Dis* 135, 267-274 (1977)

63. Sobel, J.D., Myers, P., Levison, M.E. & Kaye, D.: Comparison of bacterial and fungal adherence to vaginal exfoliated epithelial cells and human vaginal epithelial tissue culture cells. *Infect Immun* 35, 697-701 (1982)

64. Cox, F.: Prevention of group B streptococcal colonization with topically applied lipoteichoic acid in a maternal-newborn mouse model. *Pediatr Res* 16, 816-819 (1982)

65. Galask, R.P., Varner, M.W., Petzold, C.R. & Wilbur, S.L.: Bacterial attachment to the chorioamniotic membranes. *Am J Obstet Gynecol* 148, 915-928 (1984)

66. Goldschmidt, J.C., Jr. & Panos, C.: Teichoic acids of *Streptococcus agalactiae*: chemistry, cytotoxicity, and effect on bacterial adherence to human cells in tissue culture. *Infect Immun* 43, 670-677 (1984)

67. Nealon, T.J. & Mattingly, S.J.: Role of cellular lipoteichoic acids in mediating adherence of serotype III strains of group B streptococci to human embryonic, fetal, and adult epithelial cells. *Infect Immun* 43, 523-530 (1984)

68. Nealon, T.J. & Mattingly, S.J.: Kinetic and chemical analyses of the biologic significance of lipoteichoic acids in mediating adherence of serotype III group B streptococci. *Infect Immun* 50, 107-115 (1985)

69. Teti, G., Tomasello, F., Chiofalo, M.S., Orefici, G. & Mastroeni, P.: Adherence of group B streptococci to adult and neonatal epithelial cells mediated by lipoteichoic acid. *Infect Immun* 55, 3057-3064 (1987)

70. Spellerberg, B., Rozdzinski, E., Martin, S., Weber-Heynemann, J., Schnitzler, N., Lutticken, R. & Podbielski, A.: Lmb, a protein with similarities to the LraI adhesin family, mediates attachment of *Streptococcus agalactiae* to human laminin. *Infect Immun* 67, 871-878 (1999)

71. Beckmann, C., Waggoner, J.D., Harris, T.O., Tamura, G.S. & Rubens, C.E.: Identification of novel adhesins from group B streptococci by use of phage display reveals that C5a peptidase mediates fibronectin binding. *Infect Immun* 70, 2869-2876 (2002)

72. Rubens, C.E., Wessels, M.R., Heggen, L.M. & Kasper, D.L.: Transposon mutagenesis of type III group B *Streptococcus*: correlation of capsule expression with virulence. *Proc Natl Acad Sci USA* 84, 7208-7212 (1987)

73. Madoff, L.C., Hori, S., Michel, J.L., Baker, C.J. & Kasper, D.L.: Phenotypic diversity in the alpha C protein of group B streptococci. *Infect Immun* . 59, 2638-2644 (1991)

74. Schuchat, A., Deaver-Robinson, K., Plikaytis, B.D., Zangwill, K.M., Mohle-Boetani, J. & Wenger, J.D.: Multistate case-control study of maternal risk factors for neonatal group B streptococcal disease. The Active Surveillance Study Group. *Pediatr Infect Dis J* 13, 623-629 (1994)

75. Boyer, K.M. & Gotoff, S.P.: Antimicrobial prophylaxis of neonatal group B streptococcal sepsis. *Clin Perinatol* 15, 831-850 (1988)

76. Yagupsky, P., Menegus, M.A. & Powell, K.R.: The changing spectrum of group B streptococcal disease in infants: an eleven-year experience in a tertiary care hospital. *Pediatr Infect Dis J* 10, 801-808 (1991)

77. Schuchat, A. & Wenger, J.D.: Epidemiology of group B streptococcal disease. Risk factors, prevention strategies, and vaccine development. *Epidemiol Rev* 16, 374-402 (1994)

78. Jackson, L.A., Hilsdon, R., Farley, M.M., Harrison, L.H., Reingold, A.L., Plikaytis, B.D., Wenger, J.D. & Schuchat, A.: Risk factors for group B streptococcal disease in adults. *Ann Intern Med* 123, 415-420 (1995)

79. Henning, K.J., Hall, E.L., Dwyer, D.M., Billmann, L., Schuchat, A., Johnson, J.A., Harrison, L.H. & Maryland Emerging Infections, P.: Invasive group B streptococcal disease in Maryland nursing home residents. *J Infect Dis* 183, 1138-1142 (2001)

80. Ramos, E., Gaudier, F.L., Hearing, L.R., Del Valle, G.O., Jenkins, S. & Briones, D.: Group B *Streptococcus* colonization in pregnant diabetic women. *Obstet Gynecol* 89, 257-260 (1997)

81. Anthony, B.F., Okada, D.M. & Hobel, C.J.: Epidemiology of group B *Streptococcus*: longitudinal observations during pregnancy. *J Infect Dis* 137, 524-530 (1978)

82. Yow, M.D., Leeds, L.J., Thompson, P.K., Mason, E.O., Jr., Clark, D.J. & Beachler, C.W.: The natural history of group B streptococcal colonization in the pregnant woman and her offspring. I. Colonization studies. *Am J Obstet Gynecol* 137, 34-38 (1980)

83. American Academy of Pediatrics Committee on Infectious Diseases and Committee on Fetus and Newborn.: Revised guidelines for prevention of early-onset group B streptococcal (GBS) infection. *Pediatr* 99, 489-496 (1997)

84. American College of Obstetrics and Gynecologists.: ACOG committee opinion. Prevention of early-onset group B streptococcal disease in newborns. Number 173--June 1996. Committee on Obstetric Practice. *Int J Gynaecol Obstet* 54, 197-205 (1996)

85. Pearlman, M.D., Pierson, C.L. & Faix, R.G.: Frequent resistance of clinical group B streptococci isolates to clindamycin and erythromycin. *Obstet Gynecol* 92, 258-261 (1998)

86. Morales, W.J., Dickey, S.S., Bornick, P. & Lim, D.V.: Change in antibiotic resistance of group B *Streptococcus*: impact on intrapartum management. *Am J Obstet Gynecol* 181, 310-314 (1999)

87. Lin, F.Y., Azimi, P.H., Weisman, L.E., Philips, J.B., Regan, J., Clark, P., Rhoads, G.G., Clemens, J., Troendle, J., Pratt, E., Brenner, R.A. & Gill, V.: Antibiotic susceptibility profiles for group B streptococci isolated from neonates, 1995-1998. *Clin Infect Dis* 31, 76-79 (2000)

88. Andrews, J.I., Diekema, D.J., Hunter, S.K., Rhomberg, P.R., Pfaller, M.A., Jones, R.N. & Doern, G.V.: Group B streptococci causing neonatal bloodstream infection: antimicrobial susceptibility and serotyping results from SENTRY centers in the Western Hemisphere. *Am J Obstet Gynecol* 183, 859-862 (2000)

89. Manning, S.D., Pearlman, M.D., Tallman, P., Pierson, C.L. & Foxman, B.: The frequency of antibiotic resistance among group B *Streptococcus* isolated from healthy college students. *Clin Infect Dis* 33, E137-139 (2001)

90. Manning, S.D., Foxman, B., Pierson, C.L., Tallman, P., Baker, C.J. & Pearlman, M.D.: Correlates of antibiotic resistant group B *Streptococcus* isolated from pregnant women. *Obstet Gynecol* In press, (2002)

91. Stoll, B.J., Hansen, N., Fanaroff, A.A., Wright, L.L., Carlo, W.A., Ehrenkranz, R.A., Lemons, J.A., Donovan, E.F., Stark, A.R., Tyson, J.E., Oh, W., Bauer, C.R., Korones, S.B., Shankaran, S., Laptook, A.R., Stevenson, D.K., Papile, L. & Poole, K.: Changes in pathogens causing early-onset sepsis in very low-birth weight infants. *N Engl J Med* 347, 240-247 (2002)

92. Baker, C.J., Paoletti, L.C., Wessels, M.R., Guttormsen, H.K., Rench, M.A., Hickman, M.E. & Kasper, D.L.: Safety and immunogenicity of capsular polysaccharide-tetanus toxoid conjugate vaccines for group B streptococcal types Ia and Ib. *J Infect Dis* 179, 142-150 (1999)

93. Baker, C.J., Paoletti, L.C., Rench, M.A., Guttormsen, H.K., Carey, V.J., Hickman, M.E. & Kasper, D.L.: Use of capsular polysaccharide-tetanus toxoid conjugate vaccine for type II group B *Streptococcus* in healthy women. *J Infect Dis* 182, 1129-1138 (2000)

94. Selander, R.K.: Protein polymorphism and the genetic structure of natural populations of bacteria. In: Population Genetics and Molecular Evolution. Eds: Ohia, T. and Aoki, K., Springer-Verlag, Berline, Heidelberg 85-106 (1985)

95. Lancefield, R.C.: A serological differentiation of specific types of bovine hemolytic streptococci (group B). *J Exp Med* 59, 441-458 (1934)

96. Lancefield, R.C. & Hare, R.: The serological differentiation of pathogenic and non-pathogenic strains of hemolytic streptococci from parturient women. *J Exp Med* 61, 335-349 (1935)

97. Lancefield, R.C.: Two serological types of group B hemolytic streptococci with related, but not identical, type-specific substances. *J Exp Med* 67, 25-40 (1938)

98. Lancefield, R.C. & Freimer, E.H.: Type-specific polysaccharide antigens of group B streptococci. *J Hyg (Lond)* 64, 191-203 (1966)

99. Freimer, E.H.: Type-specific polysaccharide antigens of group B streptococci: II. The chemical basis for serological specificity of the type II HCI antigen. *J Exp Med* 125, 381-392 (1967)

100. Lancefield, R.C.: Cellular antigens of group B streptococci. In: Streptococci and Streptococcal Diseases: Recognition, Understanding, and Management, Eds: Wannamaker, L.W. and Matsen, J.M., Academic Press, New York 57-64 (1972)

101. Lancefield, R.C., McCarty, M. & Everly, W.N.: Multiple mouse-protective antibodies directed against group B streptococci. Special reference to antibodies effective against protein antigens. *J Exp Med* 142, 165-179 (1975)

102. Perch, B., Kjems, E. & Henrichsen: New serotypes of group B streptococci isolated from human sources. *J Clin Microbiol* 10, 109-110 (1979)

103. Jelinkova, J. & Motlova, J.: Worldwide distribution of two new serotypes of group B streptococci: type IV and provisional type V. *J Clin Microbiol* 21, 361-362 (1985)

104. Wessels, M.R., DiFabio, J.L., Benedi, V.J., Kasper, D.L., Michon, F., Brisson, J.R., Jelinkova, J. & Jennings, H.J.: Structural determination and immunochemical characterization of the type V group B *Streptococcus* capsular polysaccharide. *J Biol Chem* 266, 6714-6719 (1991)

105. von Hunolstein, C., D'Ascenzi, S., Wagner, B., Jelinkova, J., Alfarone, G., Recchia, S., Wagner, M. & Orefici, G.: Immunochemistry of capsular type polysaccharide and virulence properties of type VI *Streptococcus agalactiae* (group B streptococci). *Infect Immun* 61, 1272-1280 (1993)

106. Kogan, G., Brisson, J.R., Kasper, D.L., von Hunolstein, C., Orefici, G. & Jennings, H.J.: Structural

elucidation of the novel type VII group B Streptococcus capsular polysaccharide by high resolution NMR spectroscopy. *Carbohydr Res* 277, 1-9 (1995)

107. Kogan, G., Uhrin, D., Brisson, J.R., Paoletti, L.C., Blodgett, A.E., Kasper, D.L. & Jennings, H.J.: Structural and immunochemical characterization of the type VIII group B *Streptococcus* capsular polysaccharide. *J Biol Chem* 271, 8786-8790 (1996)

108. Kogan, G., Uhrin, D., Brisson, J.R. & *et al*..: Structure of the type VI group B streptococcus capsular polysaccharide determined by high resolution NMR spectroscopy. *J Carbohydr Chem* 13, 1071-1078 (1994)

109. Dillon, H.C., Jr., Khare, S. & Gray, B.M.: Group B streptococcal carriage and disease: a 6-year prospective study. *J Pediatr* 110, 31-36 (1987)

110. Wilkinson, H.W.: Analysis of group B streptococcal types associated with disease in human infants and adults. *J Clin Microbiol* 7, 176-179 (1978)

111. Baker, C.J.: Summary of the workshop on perinatal infections due to group B *Streptococcus*. *J Infect Dis* 136, 137-157 (1977)

112. Wilkinson, H.W., Facklam, R.R. & Wortham, E.C.: Distribution by serological type of group B streptococci isolated from a variety of clinical material over a five-year period (with special reference to neonatal sepsis and meningitis). *Infect Immun* 8, 228-235 (1973)

113. Baker, C.J. & Barrett, F.F.: Group B streptococcal infections in infants. The importance of the various serotypes. *JAMA* 230, 1158-1160 (1974).

114. Christensen, H.O., Kamper, J., Olsen, H. & Siboni, K.: Treatment of meningitis in infants caused by group B-streptococci using benzylpenicillin and streptomycin. *Dan Med Bull* 30, 416-418 (1983)

115. Mulder, C.J. & Zanen, H.C.: Neonatal group B streptococcal meningitis. *Arch Dis Child* 59, 439-443 (1984)

116. Rench, M.A. & Baker, C.J.: Neonatal sepsis caused by a new group B streptococcal serotype. *J Pediatr* 122, 638-640 (1993)

117. Lin, F.Y., Clemens, J.D., Azimi, P.H., Regan, J.A., Weisman, L.E., Philips, J.B., Rhoads, G.G., Clark, P., Brenner, R.A. & Ferrieri, P.: Capsular polysaccharide types of group B streptococcal isolates from neonates with early-onset systemic infection. *J Infect Dis* 177, 790-792 (1998)

118. Blumberg, H.M., Stephens, D.S., Licitra, C., Pigott, N., Facklam, R., Swaminathan, B. & Wachsmuth, I.K.: Molecular epidemiology of group B streptococcal infections: use of restriction endonuclease analysis of chromosomal DNA and DNA restriction fragment length polymorphisms of ribosomal RNA genes (ribotyping). *J Infect Dis* 166, 574-579 (1992)

119. Harrison, L.H., Elliott, J.A., Dwyer, D.M., Libonati, J.P., Ferrieri, P., Billmann, L. & Schuchat, A.: Serotype distribution of invasive group B streptococcal isolates in Maryland: implications for vaccine formulation. Maryland Emerging Infections Program. *J Infect Dis* 177, 998-1002 (1998)

120. Murai, T. & Mochida, T.: Further study of prevalence on group B *Streptococcus*, strain JM9 in Japan. In: Pathogenic streptococci: present and future, Eds: Lancer Publication, St. Petersburg. Russia 343-344 (1994)

121. Wagner, M., Murai, T., Wagner, B., Gunther, E. & Jelinkova, J.: JM9 strains, a new type of group B streptococci from Japan. *Zentralbl Bakteriol* 280, 488-496 (1994)

122. Butter, M.N.W. & de Moor, C.E.: *Streptococcus agalactiae* as a cause of meningitis in the newborn, and of bacteremia in adults. *Antonie Van Leeuwenhoek* 33, 439-450 (1967)

123. Haug, R.H. & Soderlund, E.: Pigment production in group B streptococci. *Acta Pathol Microbiol Scand [B]* 85, 286-288 (1977)

124. Pattison, I.H., Matthews, P.R.J. & Maxted, W.R.: Type classification by Lancefield's precipitin method of human and bovine group B streptococci isolated in Britain. *J Pathol Bacteriol* 69, 51-60 (1955)

125. Wilkinson, H.W., Thacker, L.G. & Facklam, R.R.: Nonhemolytic group B streptococci of human, bovine, and ichthyic origin. *Infect Immun* 7, 496-498 (1973)

126. Finch, L.A. & Martin, D.R.: Human and bovine group B streptococci: two distinct populations. *J Appl Bacteriol* 57, 273-278 (1984)

127. Li, J., Kasper, D.L., Ausubel, F.M., Rosner, B. & Michel, J.L.: Inactivation of the alpha C protein antigen gene, *bca*, by a novel shuttle/suicide vector results in attenuation of virulence and immunity in group B *Streptococcus*. *Proc Natl Acad Sci USA* 94, 13251-13256 (1997)

128. Lipuma, J.J.: Molecular tools for epidemiologic study of infectious diseases. *Pediatr Infect Dis J* 17, 667-675 (1998)

129. Denning, D.W., Baker, C.J., Troup, N.J. & Tompkins, L.S.: Restriction endonuclease analysis of human and bovine group B streptococci for epidemiologic study. *J Clin Microbiol* 27, 1352-1356 (1989)

130. Bingen, E., Denamur, E., Lambert-Zechovsky, N., Aujard, Y., Brahimi, N., Geslin, P. & Elion, J.: Analysis of DNA restriction fragment length polymorphism extends the evidence for breast milk transmission in *Streptococcus agalactiae* late-onset neonatal infection. *J Infect Dis* 165, 569-573 (1992)

131. Gordillo, M.E., Singh, K.V., Baker, C.J. & Murray, B.E.: Typing of group B streptococci: comparison of pulsed-field gel electrophoresis and conventional electrophoresis. *J Clin Microbiol* 31, 1430-1434 (1993)

132. Fasola, E., Livdahl, C. & Ferrieri, P.: Molecular analysis of multiple isolates of the major serotypes of group B streptococci. *J Clin Microbiol* 31, 2616-2620 (1993)

133. Musser, J.M., Mattingly, S.J., Quentin, R., Goudeau, A. & Selander, R.K.: Identification of a high-virulence clone of type III *Streptococcus agalactiae* (group B *Streptococcus*) causing invasive neonatal disease. *Proc Natl Acad Sci USA* 86, 4731-4735 (1989)

134. Ellis, S., Kotiw, M. & Garland, S.M.: Restriction endonuclease analysis of group B streptococcal isolates from two distinct geographical regions. *J Hosp Infect* 33, 279-287 (1996)

135. Ferrieri, P., Cho, D.S., Livdahl, C., Rubens, C.E. & Flores, A.E.: DNA restriction profiles of nontypable group B streptococcal clinical isolates. *Adv Exp Med Biol* 418, 343-346 (1997)

136. Elliott, J.A., Farmer, K.D. & Facklam, R.R.: Sudden increase in isolation of group B streptococci, serotype V, is not due to emergence of a new pulsed-field gel electrophoresis type. *J Clin Microbiol* 36, 2115-2116 (1998)

137. Tompkins, L.S.: The use of molecular methods in infectious diseases. *N Engl J Med* 327, 1290-1297 (1992)

138. Maslow, J.N., Mulligan, M.E. & Arbeit, R.D.: Molecular epidemiology: Application of contemporary techniques to the typing of microorganisms. *Clin Infect Dis* 17, 153-164 (1993)

139. Chaffin, D.O., Beres, S.B., Yim, H.H. & Rubens, C.E.: The serotype of type Ia and III group B streptococci is determined by the polymerase gene within the polycistronic capsule operon. *J Bacteriol* 182, 4466-4477 (2000)

140. O'Brien, T.F., Ross, D.G., Guzman, M.A., Medeiros, A.A., Hedges, R.W. & Botstein, D.: Dissemination of an antibiotic resistance plasmid in hospital patient flora. *Antimicrob Agents Chemother* 17, 537-543 (1980)

141. Hoban, D.J., Doern, G.V., Fluit, A.C., Roussel-Delvallez, M. & Jones, R.N.: Worldwide prevalence of antimicrobial resistance in *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* in the SENTRY Antimicrobial Surveillance Program, 1997-1999. *Clin Infect Dis* 32, S81-93 (2001)

142. Versalovic, J., Woods, C.R., Georghiou, P.R., Hamill, R.J. & Lupski, J.R.: DNA-based identification and epidemiologic typing of bacterial pathogens. *Arch Pathol Lab Med* 117, 1088-1098 (1993)

143. Marques, M.B., Kasper, D.L., Pangburn, M.K. & Wessels, M.R.: Prevention of C3 deposition by capsular polysaccharide is a virulence mechanism of type III group B streptococci. *Infect Immun* 60, 3986-3993 (1992)

144. Rubens, C.E., Wessels, M.R., Kuypers, J.M., Kasper, D.L. & Weiser, J.N.: Molecular analysis of two group B streptococcal virulence factors. *Semin Perinatol* 14, 22-29 (1990)

145. Klegerman, M.E., Boyer, K.M., Papierniak, C.K., Levine, L. & Gotoff, S.P.: Type-specific capsular antigen is associated with virulence in late-onset group B streptococcal type III disease. *Infect Immun* 44, 124-129 (1984)

146. Korzeniowska-Kowal, A., Witkowska, D. & Gamian, A.: Molecular mimicry of bacterial polysaccharides and their role in etiology of infectious and autoimmune diseases. *Postepy Hig Med Dosw* 55, 211-232 (2001)

147. Wilkinson, H.W. & Eagon, R.G.: Type-specific antigens of group B type Ic streptococci. *Infect Immun* 4, 596-604 (1971)

148. Bevanger, L. & Naess, A.I.: Mouse-protective antibodies against the Ibc proteins of group B streptococci. *Acta Pathol Microbiol Immunol Scand [B]* 93, 121-124 (1985)

149. Bevanger, L.: Ibc proteins as serotype markers of group B streptococci. *Acta Pathol Microbiol Immunol Scand [B]* 91, 231-234 (1983)

150. Johnson, D.R. & Ferrieri, P.: Group B streptococcal Ibc protein antigen: distribution of two determinants in wild-type strains of common serotypes. *J Clin Microbiol* 19, 506-510 (1984)

151. Lancefield, R.C.: Current knowledge of type-specific M antigens of group A streptococci. *J Immunol* 89, 307-313 (1962)

152. Baker, C.J., Webb, B.J., Kasper, D.L. & Edwards, M.S.: The role of complement and antibody in opsonophagocytosis of type II group B streptococci. *J Infect Dis* 154, 47-54 (1986)

153. Payne, N.R. & Ferrieri, P.: The relation of the Ibc protein antigen to the opsonization differences between strains of type II group B streptococci. *J Infect Dis* 151, 672-681 (1985)

154. Payne, N.R., Kim, Y.K. & Ferrieri, P.: Effect of differences in antibody and complement requirements on phagocytic uptake and intracellular killing of "c" protein-positive and -negative strains of type II group B streptococci. *Infect Immun* 55, 1243-1251 (1987)

155. Russell-Jones, G.J., Gotschlich, E.C. & Blake, M.S.: A surface receptor specific for human IgA on group B

streptococci possessing the Ibc protein antigen. *J Exp Med* 160, 1467-1475 (1984)

156. Brady, L.J. & Boyle, M.D.: Identification of non-immunoglobulin A-Fc-binding forms and low-molecular-weight secreted forms of the group B streptococcal beta antigen. *Infect Immun* 57, 1573-1581 (1989)

157. Coleman, S.E., Brady, L.J. & Boyle, M.D.: Colloidal gold immunolabeling of immunoglobulin-binding sites and beta antigen in group B streptococci. *Infect Immun* 58, 332-340 (1990)

158. Jerlstrom, P.G., Chhatwal, G.S. & Timmis, K.N.: The IgA-binding beta antigen of the c protein complex of group B streptococci: sequence determination of its gene and detection of two binding regions. *Mol Microbiol* 5, 843-849 (1991)

159. Heden, L.O., Frithz, E. & Lindahl, G.: Molecular characterization of an IgA receptor from group B streptococci: sequence of the gene, identification of a proline-rich region with unique structure and isolation of N-terminal fragments with IgA-binding capacity. *Eur J Immunol* 21, 1481-1490 (1991)

160. Jerlstrom, P.G., Talay, S.R., Valentin-Weigand, P., Timmis, K.N. & Chhatwal, G.S.: Identification of an immunoglobulin A binding motif located in the beta-antigen of the c protein complex of group B streptococci. *Infect Immun* 64, 2787-2793 (1996)

161. Madoff, L.C., Michel, J.L. & Kasper, D.L.: A monoclonal antibody identifies a protective C-protein alpha-antigen epitope in group B streptococci. *Infect Immun* 59, 204-210 (1991)

162. Michel, J.L., Madoff, L.C., Kling, D.E., Kasper, D.L. & Ausubel, F.M.: Cloned alpha and beta C-protein antigens of group B streptococci elicit protective immunity. *Infect Immun* 59, 2023-2028 (1991)

163. Kling, D.E., Gravekamp, C., Madoff, L.C. & Michel, J.L.: Characterization of two distinct opsonic and protective epitopes within the alpha C protein of the group B *Streptococcus*. *Infect Immun* 65, 1462-1467 (1997)

164. Gravekamp, C., Kasper, D.L., Paoletti, L.C. & Madoff, L.C.: Alpha C protein as a carrier for type III capsular polysaccharide and as a protective protein in group B streptococcal vaccines. *Infect Immun* 67, 2491-2496 (1999)

165. Larsson, C., Stalhammar-Carlemalm, M. & Lindahl, G.: Experimental vaccination against group B *Streptococcus*, an encapsulated bacterium, with highly purified preparations of cell surface proteins Rib and alpha. *Infect Immun* 64, 3518-3523 (1996)

166. Baker, C.J.: Immunization to prevent group B streptococcal disease: victories and vexations. *J Infect Dis* 161, 917-921 (1990)

167. Insel, R.A.: Maternal immunization to prevent neonatal infections. *N Engl J Med* 319, 1219-1220 (1988)

168. Wilkinson, H.W.: Comparison of streptococcal R antigens. *Appl Microbiol* 24, 669-670 (1972)

169. Flores, A.E. & Ferrieri, P.: Molecular species of R-protein antigens produced by clinical isolates of group B streptococci. *J Clin Microbiol* 27, 1050-1054 (1989)

170. Stalhammar-Carlemalm, M., Stenberg, L. & Lindahl, G.: Protein rib: a novel group B streptococcal cell surface protein that confers protective immunity and is expressed by most strains causing invasive infections. *J Exp Med* 177, 1593-1603 (1993)

171. Stalhammar-Carlemalm, M., Areschoug, T., Larsson, C. & Lindahl, G.: Cross-protection between group A and group B streptococci due to cross-reacting surface proteins. *J Infect Dis* 182, 142-149 (2000)

172. Lachenauer, C.S. & Madoff, L.C.: A protective surface protein from type V group B streptococci shares N-terminal sequence homology with the alpha C protein. *Infect Immun* 64, 4255-4260 (1996)

173. Kvam, A.I., Bevanger, L.S. & Iversen, O.-J.: Characterization of a surface protein of group B streptococci resembling the a antigen of the C proteins. In: Pathogenic streptococci: present and future. Eds: Totolian, A., Lancer Publication, St. Petersburg, Russia (1993)

174. Areschoug, T., Stalhammar-Carlemalm, M., Larsson, C. & Lindahl, G.: Group B streptococcal surface proteins as targets for protective antibodies: identification of two novel proteins in strains of serotype V. *Infect Immun* 67, 6350-6357 (1999)

175. Erdogan, S., Fagan, P.K., Talay, S.R., Rohde, M., Ferrieri, P., Flores Alia, C., Guzman, C.A., Walker, M.J. & Chhatwal, G.S.: Molecular analysis of group B protective surface protein, a new cell surface protective antigen of group B streptococci. *Infect Immun* 70, 803-811 (2002)

176. Suvorov, A.N., Flores, A.E. & Ferrieri, P.: Cloning of the glutamine synthetase gene from group B streptococci. *Infect Immun* 65, 191-196 (1997)

177. Pancholi, V. & Fischetti, V.A.: Alpha-enolase, a novel strong plasmin(ogen) binding protein on the surface of pathogenic streptococci. *J Biol Chem* 273, 14503-14515 (1998)

178. Hamel, J., Brodeur, B.R., Martin, D. & Rioux, C.R. Streptococcal heat shock proteins members of the Hsp70 family. U.S. patent WO9640928 (December 1996)

179. Brodeur, B.R., Boyer, M., Charlebois, I., Hamel, J., Couture, F., Rioux, C.R. & Martin, D.: Identification of group B streptococcal Sip protein, which elicits cross-protective immunity. *Infect Immun* 68, 5610-5618 (2000)

180. Chun, C.S., Brady, L.J., Boyle, M.D., Dillon, H.C. & Ayoub, E.M.: Group B streptococcal C protein-associated antigens: association with neonatal sepsis. *J Infect Dis* 163, 786-791 (1991)

181. Kvam, A.I., Efstratiou, A., Bevanger, L., Cookson, B.D., Marticorena, I.F., George, R.C. & Maeland, J.A.: Distribution of serovariants of group B streptococci in isolates from England and Norway. *J Med Microbiol* 42, 246-250 (1995)

182. Gray, B.M. & Pritchard, D.G.: Phase variation in the pathogenesis of group B streptococcal infections. *Zbl Bakt* Suppl. 22, 452-454 (1992)

183. Sellin, M., Linderholm, M., Norgren, M. & Hakansson, S.: Endocarditis caused by a group B *Streptococcus* strain, type III, in a nonencapsulated phase. *J Clin Microbiol* 30, 2471-2473 (1992)

184. Sellin, M., Hakansson, S. & Norgren, M.: Phase-shift of polysaccharide capsule expression in group B streptococci, type III. *Microb Pathog* 18, 401-415 (1995)

185. Selander, R.K., Musser, J.M., Caugant, D.A., Gilmour, M.N. & Whittam, T.S.: Population genetics of pathogenic bacteria. *Microb Pathog* 3, 1-7 (1987)

186. Granlund, M., Oberg, L., Sellin, M. & Norgren, M.: Identification of a novel insertion element, IS*1548*, in group B streptococci, predominantly in strains causing endocarditis. *J Infect Dis* 177, 967-976 (1998)

187. Hauge, M., Jespersgaard, C., Poulsen, K. & Kilian, M.: Population structure of *Streptococcus agalactiae* reveals an association between specific evolutionary lineages and putative virulence factors but not disease. *Infect Immun* 64, 919-925 (1996)

188. Mattingly, S.J., Maurer, J.J., Eskew, E.K. & Cox, F.: Identification of a high-virulence clone of serotype III *Streptococcus agalactiae* by growth characteristics at 40 degrees C. *J Clin Microbiol* 28, 1676-1677 (1990)

189. Quentin, R., Huet, H., Wang, F.S., Geslin, P., Goudeau, A. & Selander, R.K.: Characterization of *Streptococcus agalactiae* strains by multilocus enzyme genotype and serotype: identification of multiple virulent clone families that cause invasive neonatal disease. *J Clin Microbiol* 33, 2576-2581 (1995)

190. Helmig, R., Uldbjerg, N., Boris, J. & Kilian, M.: Clonal analysis of *Streptococcus agalactiae* isolated from infants with neonatal sepsis or meningitis and their mothers and from healthy pregnant women. *J Infect Dis* 168, 904-909 (1993)

191. Weber, S., Pfaller, M.A. & Herwaldt, L.A.: Role of molecular epidemiology in infection control. *Infect Dis Clin North Am* 11, 257-277 (1997)

192. Rabkin, C.S., Jarvis, W.R., Anderson, R.L. Govan, J., Klinger, J. LiPuma, J., Martone, W.J., Monteil, H., Richard, C., Shigeta, S., *et al.*: *Psuedomonas cepacia* typing systems: collaborative study to assess their potential in epidemiologic investigations. *Rev Infect Dis* 11, 600-607 (1989)

193. Musser, J.M.: Molecular population analysis of emerging bacterial pathogens:selected insights. *Emerg Infect Dis* 2, 1-17 (1996)

194. El-Solh, N., Bouanchaud, D.H., Horodniceanu, T., Roussel, A. & Chabbert, Y.A.: Molecular studies and possible relatedness between R plasmids from groups B and D streptococci. *Antimicrob Agents Chemother* 14, 19-23 (1978)

195. Stringer, J.: The development of a phage-typing system for group-B streptococci. *J Med Microbiol* 13, 133-143 (1980)

196. Boyer, K.M., Vogel, L.C., Gotoff, S.P., Gadzala, C.A., Stringer, J. & Maxted, W.R.: Nosocomial transmission of bacteriophage type 7/11/12 group B streptococci in a special care nursery. *Am J Dis Child* 134, 964-966 (1980)

197. Noya, F.J., Rench, M.A., Metzger, T.G., Colman, G., Naidoo, J. & Baker, C.J.: Unusual occurrence of an epidemic of type Ib/c group B streptococcal sepsis in a neonatal intensive care unit. *J Infect Dis* 155, 1135-1144 (1987)

198. Weems, J.J., Jr., Jarvis, W.R. & Colman, G.: A cluster of late onset group B streptococcal infections in low birth weight premature infants: no evidence for horizontal transmission. *Pediatr Infect Dis* 5, 715-717 (1986)

199. Spellerberg, B., Pohl, B., Haase, G., Martin, S., Weber-Heynemann, J. & Lutticken, R.: Identification of genetic determinants for the hemolytic activity of *Streptococcus agalactiae* by IS*S1* transposition. *J Bacteriol* 181, 3212-3219 (1999)

200. Hassan, A.A., Abdulmawjood, A., Yildirim, A.O., Fink, K., Lammler, C. & Schlenstedt, R.: Identification of streptococci isolated from various sources by determination of *cfb* gene and other CAMP-factor genes. *Can J Microbiol* 46, 946-951 (2000)

201. Michel, J.L., Madoff, L.C., Olson, K., Kling, D.E., Kasper, D.L. & Ausubel, F.M.: Large, identical, tandem repeating units in the C protein alpha antigen gene, *bca*, of group B streptococci. *Proc Natl Acad Sci USA* 89, 10060-10064 (1992)

202. Madoff, L.C., Michel, J.L., Gong, E.W., Kling, D.E. & Kasper, D.L.: Group B streptococci escape host immunity by deletion of tandem repeat elements of the alpha C protein. *Proc Natl Acad Sci USA* 93, 4131-4136 (1996)

203. Gravekamp, C., Horensky, D.S., Michel, J.L. & Madoff, L.C.: Variation in repeat number within the alpha C protein of group B streptococci alters antigenicity and protective epitopes. *Infect Immun* 64, 3576-3583 (1996)

204. Gravekamp, C., Rosner, B. & Madoff, L.C.: Deletion of repeats in the alpha C protein enhances the pathogenicity of group B streptococci in immune mice. *Infect Immun* 66, 4347-4354 (1998)

205. Berner, R., Bender, A., Rensing, C., Forster, J. & Brandis, M.: Low prevalence of the immunoglobulin-A-binding beta antigen of the C protein among *Streptococcus agalactiae* isolates causing neonatal sepsis. *Eur J Clin Microbiol Infect Dis* 18, 545-550 (1999)

206. Maeland, J.A., Brakstad, O.G., Bevanger, L. & Kvam, A.I.: *Streptococcus agalactiae* beta gene and gene product variations. *J Med Microbiol* 46, 999-1005 (1997)

207. Wastfelt, M., Stalhammar-Carlemalm, M., Delisse, A.M., Cabezon, T. & Lindahl, G.: Identification of a family of streptococcal surface proteins with extremely repetitive structure. *J Biol Chem* 271, 18892-18897 (1996)

208. Suvorov, A., Dmitriev, A., Ustinovitch, I., Schalen, C. & Totolian, A.A.: Molecular analysis of clinical group B streptococcal strains by use of alpha and beta gene probes. *FEMS Immunol Med Microbiol* 17, 149-154 (1997)

209. Kong, F., Gowan, S., Martin, D., James, G. & Gilbert, G.L.: Molecular profiles of group B streptococcal surface protein antigen genes: Relationship to molecular serotypes. *J Clin Microbiol* 40, 620-626 (2002)

210. Reinscheid, D.J., Gottschalk, B., Schubert, A., Eikmanns, B.J. & Chhatwal, G.S.: Identification and molecular analysis of PcsB, a protein required for cell wall separation of group B *Streptococcus*. *J Bacteriol* 183, 1175-1183 (2001)

211. Poyart, C., Pellegrini, E., Gaillot, O., Boumaila, C., Baptista, M. & Trieu-Cuot, P.: Contribution of Mn-cofactored superoxide dismutase (SodA) to the virulence of *Streptococcus agalactiae*. *Infect Immun* 69, 5098-5106 (2001)

212. Tamura, G.S., Nittayajarn, A. & Schoentag, D.L.: A glutamine transport gene, *glnQ*, is required for fibronectin adherence and virulence of group B streptococci. *Infect Immun* 70, 2877-2885 (2002)

213. Spellerberg, B., Rozdzinski, E., Martin, S., Weber-Heynemann, J. & Lutticken, R.: *rgf* encodes a novel two-component signal transduction system of *Streptococcus agalactiae. Infect Immun* 70, 2434-2440 (2002)

214. Hughes, M.J.G., Moore, J.C., Lane, J.D., Wilson, R., Pribul, P.K., Younes, Z.N., Dobson, R.J., Everest, P., Reason, A.J., Redfern, J.M., Greer, F.M., Paxton, T., Panico, M., Morris, H.R., Feldman, R.G. & Santangelo, J.D.: Identification of major outer surface proteins of *Streptococcus agalactiae*. *Infect Immun* 70, 1254-1259 (2002)

215. Milligan, T.W., Baker, C.J., Straus, D.C. & Mattingly, S.J.: Association of elevated levels of extracellular neuraminidase with clinical isolates of type III group B streptococci. *Infect Immun* 21, 738-746 (1978)

216. Duran-Reynals, F.: Tissue permeability and spreading factors in infection. *Bacteriol Rev* 6, 197-252 (1942)

217. Spellerberg, B., Martin, S., Franken, C., Berner, R. & Lutticken, R.: Identification of a novel insertion sequence element in *Streptococcus agalactiae*. *Gene* 241, 51-56 (2000)

218. Sanchez-Beato, A.R., Garcia, E., Lopez, R. & Garcia, J.L.: Identification and characterization of IS*1381*, a new insertion sequence in *Streptococcus pneumoniae*. *J Bacteriol* 179, 2459-2463 (1997)

219. Tamura, G.S., Herndon, M., Przekwas, J., Rubens, C.E., Ferrieri, P. & Hillier, S.L.: Analysis of restriction fragment length polymorphisms of the insertion sequence IS*1381* in group B Streptococci. *J Infect Dis* 181, 364-368 (2000)

220. Takahashi, S., Adderson, E.E., Nagano, Y., Nagano, N., Briesacher, M.R. & Bohnsack, J.F.: Identification of a highly encapsulated, genetically related group of invasive type III group B streptococci. *J Infect Dis* 177, 1116-1119 (1998)

221. Denning, D.W., Bressack, M., Troup, N.J. & Tompkins, L.S.: Infant with two relapses of group B streptococcal sepsis documented by DNA restriction enzyme analysis. *Pediatr Infect Dis J* 7, 729-732 (1988)

222. Bohnsack, J.F., Takahashi, S., Detrick, S.R., Pelinka, R., Hammitt, L.L., Aly, A.A., Whiting, A.A. & Adderson, E.E.: Phylogenetic classification of serotype III group B streptococci based on *hylB* gene analysis and DNA sequences specific to RDP type III. *J Infect Dis* 183, (2001)

223. Bohnsack, J.F., Whiting, A.A., Bradford, R.D., Van Frank, B.K., Takahashi, S. & Adderson, E.E.: Long-range mapping of the *Streptococcus agalactiae* phylogenetic lineage restriction digest pattern type III-3 reveals clustering of virulence genes. *Infect Immun* 70, 134-139 (2002)

224. Huet, H., Martin, C., Geslin, P., Grimont, F. & Quentin, R.: Ribotyping of *Streptococcus agalactiae* strains isolated from vaginas of asymptomatic women. *Res Microbiol* 144, 457-465 (1993)

225. Gordillo, M.E., Singh, K.V. & Murray, B.E.: Comparison of ribotyping and pulsed-field gel electrophoresis for subspecies differentiation of strains of *Enterococcus faecalis*. *J Clin Microbiol* 31, 1570-1574 (1993)

226. Prevost, G., Jaulhac, B. & Piemont, Y.: DNA fingerprinting by pulsed-field gel electrophoresis is more effective than ribotyping in distinguishing among methicillin-resistant *Staphylococcus aureus* isolates. *J Clin Microbiol* 30, 967-973 (1992)

227. Denamur, E., Picard, B., Goullet, P., Bingen, E., Lambert, N. & Elion, J.: Complexity of *Pseudomonas aeruginosa* infection in cystic fibrosis: rDNA restriction fragment length polymorphism analysis. *Epidemiol. Infect* 106, 531-539 (1991)

228. Morgan, M.G., Stuart, C., Leanord, A.T., Enright, M. & Cole, G.F.: *Citrobacter diversus* brain abcess: case reports and molecular epidemiology. *J Med Microbiol* 36, 273-278 (1992)

229. Chatellier, S., Huet, H., Kenzi, S., Rosenau, A., Geslin, P. & Quentin, R.: Genetic diversity of rRNA operons of unrelated *Streptococcus agalactiae* strains isolated from cerebrospinal fluid of neonates suffering from meningitis. *J Clin Microbiol* 34, 2741-2747 (1996)

230. Arbeit, R.D.: Laboratory procedures for the epidemiologic analysis of microorganisms. In: Manual of Clinical Microbiology. Eds: Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C. and Yolken, R.H., American Society for Microbiology, Washington, D. C. 116-137 (1999)

231. Sader, H.S., Hollis, R.J. & Pfaller, M.A.: The use of molecular techniques in the epidemiology of control of infectious diseases. *Clin Lab Med* 15, 407-431 (1995)

232. Rolland, K., Marois, C., Siquier, V., Cattier, B. & Quentin, R.: Genetic features of *Streptococcus agalactiae* strains causing severe neonatal infections, as revealed by pulsed-field gel electrophoresis and *hylB* gene analysis. *J Clin Microbiol* 37, 1892-1898 (1999)

233. Green, P.A., Singh, K.V., Murray, B.E. & Baker, C.J.: Recurrent group B streptococcal infections in infants: clinical and microbiologic aspects. *J Pediatr* 125, 931-938 (1994)

234. Nagano, Y., Nagano, N., Takahashi, S., Murono, K., Fujita, K., Taguchi, F. & Okuwaki, Y.: Restriction endonuclease digest patterns of chromosomal DNA from group B beta-haemolytic streptococci. *J Med Microbiol* 35, 297-303 (1991)

235. Chatellier, S., Ramanantsoa, C., Harriau, P., Rolland, K., Rosenau, A. & Quentin, R.: Characterization of *Streptococcus agalactiae* strains by randomly amplified polymorphic DNA analysis. *J Clin Microbiol* 35, 2573-2579 (1997)

236. Tenover, F.C., Arbeit, R.D., Goering, R.V., Mickelson, P.A., Murray, B.E., Persing, D.H. Swaminathan, B.: Interpreting chormosomal DNA patterns produced by pulsed-field gel electrophoresis: criteria for bacterial typing. *J Clin Microbiol* 33, 2233-2239 (1995)

237. Williams, J.G.K., Kubelik, A.R., Livak, J.A., Rafalski, J.A. & Tingey, S.V.: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res* 18, 6531-3535 (1990)

238. Welsh, J. & McClelland, M.: Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res* 18, 7213-7218 (1990)

239. Limansky, A.S., Guardati, M.C., Sutich, E.G., Toresani, I.E., Bogado, I.E., Joannas, G.M. & Viale, A.M.: Characterization of clinical isolates of *Streptococcus agalactiae* by random amplified polymorphic DNA using degenerate oligonucleotides. *Medicina (B Aires)* 55, 681-684 (1995)

240. Wang, G., Whittam, T.S., Berg, C.M. & Berg, D.E.: RAPD (arbitrary primer) PCR is more sensitive than multilocus enzyme electrophoresis for distinguishing related bacterial strains. *Nucleic Acids Res* 21, 5930-5933 (1993)

241. Culebras, E., Rodriguez-Avail, I., C., B., Redondo, M. & Picazo, J.J.: Macrolide and tetracycline resistant and molecular relationships of clinical strains of *Streptococcus agalactiae. Antimicrob Agents Chemother* 46, 1574-1576 (2002)

242. Paoletti, L.C., Peterson, D.L., Legmann, R. & Collier, R.J.: Preclinical evaluation of group B streptococcal polysaccharide conjugate vaccines prepared with a modified diphtheria toxin and a recombinant duck hepatitis B core antigen. *Vaccine* 20, 370-376 (2001)

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