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# Serine-Rich Repeat Proteins and Pili Promote *Streptococcus agalactiae* Colonization of the Vaginal Tract[▽]

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***Streptococcus agalactiae*** **(group B streptococcus [GBS]) is a Gram-positive bacterium found in the female rectovaginal tract and is capable of producing severe disease in susceptible hosts, including newborns and pregnant women. The vaginal tract is considered a major reservoir for GBS, and maternal vaginal colonization poses a significant risk to the newborn; however, little is known about the specific bacterial factors that promote GBS colonization and persistence in the female reproductive tract. We have developed *in vitro* models of GBS interaction with the human female cervicovaginal tract using human vaginal and cervical epithelial cell lines. Analysis of isogenic mutant GBS strains deficient in cell surface organelles such as pili and serine-rich repeat (Srr) proteins shows that these factors contribute to host cell attachment. As Srr proteins are heavily glycosylated, we confirmed that carbohydrate moieties contribute to the effective interaction of Srr-1 with vaginal epithelial cells. Antibody inhibition assays identified keratin 4 as a possible host receptor for Srr-1. Our findings were further substantiated in an *in vivo* mouse model of GBS vaginal colonization, where mice inoculated with an Srr-1-deficient mutant exhibited decreased GBS vaginal persistence compared to those inoculated with the wild-type (WT) parental strain. Furthermore, competition experiments in mice showed that WT GBS exhibited a significant survival advantage over the Δ*pilA* or Δ*srr-1* mutant in the vaginal tract. Our results suggest that these GBS surface proteins contribute to vaginal colonization and may offer new insights into the mechanisms of vaginal niche establishment.**

Group B streptococcus (GBS) is the leading cause of neonatal meningitis and sepsis in the developed world and also causes serious invasive infections in certain adult populations (54). GBS can be isolated from the rectovaginal tracts of up to 30% of women (16, 38), and it can be transmitted to infants during birth through the aspiration of vaginal fluids or cross the placental barrier *in utero* (7, 18). GBS neonatal infection is divided into two categories, early-onset (<7 days old) and late-onset (7 to 90 days old) disease. Due to the serious nature of GBS infection, pregnant women in the United States are routinely screened for GBS vaginal colonization late in the third trimester of pregnancy; a positive test results in the administration of antibiotics during birth to reduce the risk of GBS transfer to the newborn. Despite this intervention, the incidence of early-onset GBS infection in the United States remains at 1 in 3,000 live births, corresponding to approximately 1,200 infected infants per year (54). There is also evidence that infection rates are much higher among some ethnic groups and in infants delivered at <37 weeks of gestation (42, 43, 54, 62). Additionally, antibiotic prophylaxis does not prevent late-onset disease.

Most women are intermittently asymptomatically colonized by GBS in the genitourinary tract (19); however, colonization poses a significant risk to both mother and fetus during pregnancy and birth (34). Bacteria colonize the mucosal layer of the lower vaginal vault and can ascend higher into the ecto-and endocervical cell layers. The normal vaginal microbiota is dynamic and can be influenced by diverse factors such as hormone levels, pH, age, and ethnicity (37). To persist in this changing environment, GBS most likely elaborates factors to facilitate attachment to the vaginal epithelium. Surface-associated organelles such as pili and serine-rich repeat (Srr) proteins are associated with GBS attachment to human cells (10, 22, 41, 53). Streptococcal and staphylococcal Srr proteins contain a characteristic LPXTG anchoring motif that is recognized by a sortase enzyme responsible for cell wall linkage. GBS Srr is secreted by the SecA2 system and then anchored to the cell wall by housekeeping sortase A (27). The GBS Srr protein, like its homologues PsrP in *Streptococcus pneumoniae* and GspB in *Streptococcus gordonii*, has a very high molecular weight and undergoes extensive posttranslational modification by way of glycosylation (53). These surface proteins are known to interact with host factors, facilitating cellular adherence and, in some cases, invasion (53). There are two recognized Srr loci in GBS strains that are functionally similar but share less than 20% identity. Srr-1 is found in the majority of strains belonging to the Ia, Ib, and V and certain III capsular serotype groups, while Srr-2 is unique to serotype III-3 strains and multilocus sequencing type 17 strains (44). Strains from capsular serotypes Ia, III, and V are all commonly associated with neonatal infection, and therefore both Srr-1 and Srr-2 are likely to be important for disease and colonization.

Like Srr proteins, GBS pilus proteins contain the cell wall-anchoring motif, which is recognized by specific sortases for

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TABLE 1. Bacterial strains used in this study

| Species and strain | Description | Reference or source |
|---|---|---|
| *S. agalactiae* (GBS) | | |
| A909 | Wild-type clinical isolate, serotype Ia | 21 |
| NCTC 10/84 | Wild-type clinical isolate, 1169-NT1, serotype V | 59 |
| COH1 | Wild-type clinical isolate, serotype III | 60 |
| NEM316 | Wild-type clinical isolate, serotype III | 14 |
| 515 | Wild-type clinical isolate, serotype Ia | 57 |
| Δ*srr-1*::*cat* (Δ*srr-1*) strain | NCTC 10/84 with in-frame allelic replacement of *srr-1* with the chloramphenicol acetyltransferase gene (*cat*) | 53 |
| Δ*pilA*::*cat* (Δ*pilA*) strain | NCTC 10/84 with in-frame allelic replacement of *pilA* with the chloramphenicol acetyltransferase gene (*cat*) | 22 |
| Δ*pilB*::*cat* (Δ*pilB*) strain | NCTC 10/84 with in-frame allelic replacement of *pilB* with the chloramphenicol acetyltransferase gene (*cat*) | 22 |
| Δ*iagA*::*cat* (Δ*iagA*) strain | COH1 with in-frame allelic replacement of *iagA* with the chloramphenicol acetyltransferase gene (*cat*) | 9 |
| Δ*srr-2*::*cat* (Δ*srr-2*) strain | COH1 with in-frame allelic replacement of *srr-2* with the chloramphenicol acetyltransferase gene (*cat*) | This study |
| Δ*srr-1* p*srr-1* strain | Δ*srr-1* strain expressing *srr-1* in pDCerm | 53 |
| Δ*pilA* p*pilA* strain | Δ*pilA* strain expressing *pilA* in pDCerm | 22 |
| Δ*pilA srr-1*::pHY304 strain | Δ*pilA* strain with disruption of *srr-1* by plasmid pHY304 insertion | This study |
| NEM316 *pilA*::pHY304 | NEM316 with disruption of *pilA* by plasmid pHY304 insertion | 2 |
| 515 *pilA*::pHY304 | 515 with disruption of *pilA* by plasmid pHY304 insertion | 2 |
| *B. thuringiensis* HD1 | *Bacillus thuringiensis* subsp. *kurstaki*, wild-type isolate | *Bacillus* Stock Center |
| *Escherichia coli* TOP10 | F$^-$ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) ϕ80*lacZ*ΔM15 Δ*lacX74 deoR recA1 ara*Δ*139* Δ(*ara-leu*)*7697 galU galK rpsL* (Str$^r$) *endA1 nupG* | Invitrogen |

correct cleavage and membrane anchoring. GBS pili are multimeric structures consisting of the three pilin proteins PilA, PilB, and PilC (23). The majority of the pilus backbone is made up of PilB subunits, with a PilA subunit at the terminus of each pilus and a PilC protein at the base (10, 22, 23, 39). PilB has previously been shown to be essential for human cell invasion, while PilA plays a role in cellular attachment (10, 22). Three pilus loci, pilus island 1 (PI-1), PI-2a, and PI-2b, have been described in GBS; all sequenced strains contain at least one locus and several strains contain more than one locus (39), suggesting that pilus function in GBS is highly conserved.

As Srr and pilus proteins are known to mediate host cell attachment by GBS and other streptococcal species, we hypothesized that these proteins may promote cervicovaginal epithelial cell attachment and colonization of the vagina. Here we confirm that GBS readily adheres to cells of the vaginal tract and that the pilus tip adhesin, PilA (from PI-2a), and Srr proteins contribute to attachment, both *in vitro* and in a mouse model of GBS vaginal colonization. These results represent the first identification of GBS factors required for host colonization in the female vaginal tract.

## MATERIALS AND METHODS

**Bacterial strains and growth conditions.** GBS wild-type (WT) clinical isolates NCTC 10/84 (1169-NT1; ATCC 49447) (serotype V) (59), COH1 (serotype III) (60), A909 (serotype Ia) (21), NEM316 (serotype III) (14), and 515 (serotype Ia) (57) (a comprehensive list of strains is given in Table 1) were used in this study. GBS was grown in Todd-Hewitt broth (THB) (Hardy Diagnostics) at 37°C. GBS Δ*srr-1*::*cat* (referred to as Δ*srr-1*), Δ*pilA*::*cat* (referred to as Δ*pilA*), Δ*pilB*::*cat* (referred to as Δ*pilB*) (22, 53), Δ*iag*::*cat* (referred to as Δ*iagA*) (9), and Δ*srr-2*::*cat* (referred to as Δ*srr-2*) (this study) mutant strains were selected with 2 μg ml$^{-1}$ chloramphenicol (Cm). The Srr-1 and PilA complementation vectors, p*srr-1* and p*pilA*, respectively, were maintained with 5 μg ml$^{-1}$ erythromycin (Erm) (53). The *pilA* insertional mutants (NEM316 and 515 parent) (2) were maintained with 5 μg ml$^{-1}$ Erm. The Δ*pilA srr-1*::pHY304 double mutant was grown with similar concentrations of Cm and Erm. *Bacillus thuringiensis* was cultured on brain heart infusion (BHI) medium and *Escherichia coli* in LB at 37°C.

**Cell lines.** Immortalized human vaginal (VK2/E6E7), ectocervical (Ect1/E6E7), and endocervical (End1/E6E7) epithelial cell lines were obtained from the American Type Culture Collection (ATCC CRL-2616, ATCC CRL-2614, and ATCC CRL-2615, respectively) (12). All cells lines were maintained at 37°C with 5% $CO_2$ in keratinocyte serum-free medium (KSFM) (Invitrogen) with 0.1 ng ml$^{-1}$ human recombinant epidermal growth factor (EGF), 0.05 mg ml$^{-1}$ bovine pituitary extract, and additional calcium chloride (final concentration, 0.4 nM).

**Targeted mutagenesis and complementation.** The Δ*srr-1*, Δ*pilA*, Δ*pilB*, and Δ*iagA* deletion mutant strains, as well as insertional *pilA* mutants of other GBS serotypes, have been described previously; all strains exhibit growth characteristics and chain lengths similar to those of the parental strain (2, 9, 22, 53). Complementation and the generation of complementation constructs for *pilA* and *srr-1* have been described previously (22, 53). PCR was used to generate an in-frame substitution of the *srr-2* gene with the chloramphenicol acetyltransferase gene (*cat*) using a method described previously (9). Briefly, 770 bp of sequence immediately upstream of *srr-2* was amplified with the primers *srr-2*upF (GTC TTT GCA CCC ATG CAG GAC) and *srr-2*upR+cat (GGT GGT ATA TCC AGT GAT TTT TTT CTC CAT TTT TCC TCC ATA TTT AAA AAA TAA TGC), and 801 bp immediately downstream of *srr-2* was amplified with the primers *srr-2*downF+cat (TAC TGC GAT GAG TGG CAG GGC GGG GCG TAA TAA TTT TCA AAG TGA TAT TTA G) and *srr-2*downR (GGT ATA CGA TAT TCT GGC CTA). The *srr-2*upR+cat and *srr-2*downF+cat primers were constructed with 25-bp 5′ extensions corresponding to the 5′ and 3′ ends of the *cat* gene, respectively. The upstream and downstream PCR products were then combined with the 650-bp amplicon of the complete *cat* gene as templates in a second round of PCR using primers *srr-2*upF and *srr-2*downR. The resultant PCR amplicon, containing an in-frame substitution of *srr-2* with *cat*, was subcloned into temperature-sensitive vector pHY304, and allelic exchange mutagenesis in COH1 was performed as described previously (36) to generate the stable mutant COH1 Δ*srr-2*::*cat*. Precise in-frame allelic replacement of *srr-2* with *cat* in the COH1 chromosome was confirmed by PCR, restriction digestion, and sequence analysis.

Mutation of the *srr-1* gene in the Δ*pilA* mutant to create a Δ*pilA srr-1*::pHY304 double mutant was achieved using insertional duplication mutagenesis as described previously (36). Briefly, 590 bp of the *srr-1* gene was amplified using primers 5′-CGTGAAGCTTGCAGTTTGGAAACTTTGGTG-3′ and 5′-TCAG CTGCAGGTTGAACTCTAGCGGTCGTTGC-3′. The resultant fragment was

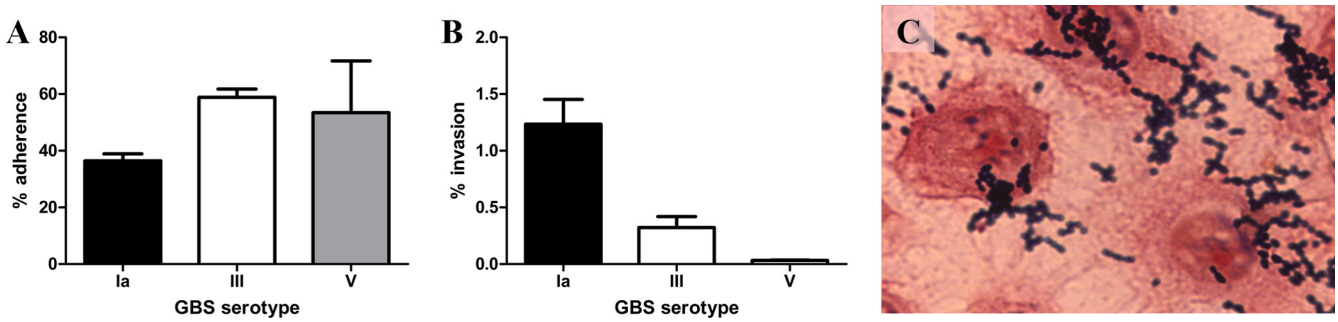


FIG. 1. GBS interaction with vaginal epithelium. (A and B) Adherence (A) and invasion (B) of VK2/E6E7 cells by representative GBS serotypes Ia, III, and V. Data are expressed as the total cell-associated (adherent) or intracellular CFU recovered compared to the input inoculum (MOI of 1; $\sim 1 \times 10^5$ CFU). All experiments were repeated at least three times in triplicate wells; data from a representative experiment are shown. (C) Micrograph of Gram-stained VK2/E6E7 vaginal epithelial cells following inoculation with GBS strain NCTC10/84 (serotype V) and multiple (6) washes. Adherent organisms are visualized. Magnification, ×1,000.

cloned into pHY304 and transformed into the Δ*pilA* mutant strain. Insertional mutants were selected with Erm, and plasmid integration was confirmed by PCR analysis using primers homologous to plasmid sequences and to sequences upstream of the point of insertion.

***In vitro* cell assays.** GBS adherence and invasion assays were performed as described previously for other cell types with minor modifications (9, 29). Briefly, cells were grown to confluence in 24-well tissue culture-treated plates and then washed prior to the addition of bacteria. Bacteria were grown to mid-log phase and then added to cells at a multiplicity of infection (MOI) of 1. To determine the total number of surface-associated (adherent) organisms, monolayers and bacteria were incubated for 30 min. Monolayers were washed 6 times with phosphate-buffered saline (PBS) to remove nonadherent organisms, and then lysis was performed by the addition of trypsin-EDTA plus Triton X-100 and vigorous pipetting to liberate adherent bacteria. Serial dilutions of this lysate were plated on THB agar plates to enumerate bacterial CFU. The total cell-associated CFU percentage was calculated as (total CFU recovered from the well/CFU of the original input inoculum) × 100%. To determine the number of intracellular bacteria, plates were incubated for 2 h, the monolayers were washed, and 0.5 ml KSFM containing 100 μg gentamicin and 5 μg penicillin was added and left for an additional 2 h. Monolayers were again washed 3 times with PBS and lysed as described above, and the number of invasive bacteria was quantified by serial dilution plating. Lectin binding assays were performed as described for the adherence assay with the following modifications. Prior to addition to cell monolayers, bacteria were incubated for 30 min with 10 μg $ml^{-1}$ wheat germ agglutinin (WGA), peanut agglutinin (PNA), or concanavalin A (ConA) (Vector Laboratories). KSFM adjusted to pH 4.5 with concentrated HCl was used for all lectin binding assays. A 30-min adherence assay was then carried out as described above. For antibody inhibition assays, confluent cell monolayers were incubated with monoclonal anti-cytokeratin peptide 4 (clone 6B10) (Sigma) for 60 min prior to the addition of bacteria (MOI = 0.1). A mouse IgG1 isotope control (R & D Systems) was used as a negative control.

**Microscopy.** Cell monolayers were propagated on glass coverslips within 24-well plates. Following a standard adherence assay (MOI = 10), monolayers were washed 5 × with Dulbecco's PBS (DPBS) and the coverslips removed from the trays. Coverslips were air dried, heat fixed, and then subjected to a standard Gram stain protocol. Images were taken using a Zeiss upright microscope with an attached Axiocam Icc3 camera.

***In vivo* mouse model of GBS vaginal colonization.** All mouse work was approved by the Office of Lab Animal Care at San Diego State University and carried out using accepted veterinary standards. Outbred female CD1 mice, 8 to 12 weeks of age, were used for all assays. Intraperitoneal injection of 0.5 mg β-estradiol valerate suspended in sesame oil (Sigma) on day 0 was carried out to synchronize estrus in all mice and optimize bacterial colonization (5). On day 3 postinjection, $\sim 1 \times 10^7$ CFU (10-μl total volume) GBS was introduced to the vaginal cavity of anesthetized mice. On successive days the cervicovaginal vault of each mouse was swabbed with ultrafine calcium alginate-tipped swabs and the recovered bacteria enumerated by serial dilution plating. WT GBS was identified by orange/red pigmentation of colonies on THB medium or on CHROMagar StrepB agar (DRG International Inc.) (35). Duplicate plating of samples on THB medium supplemented with 5% sheep blood allowed further identification of GBS by the presence of beta-hemolysis. Mutant strains were selected with the appropriate antibiotics.

**Statistical analysis.** GraphPad Prism version 4.03 was used for statistical analyses. Differences in adherence, invasion, and number of bacteria recovered from the mouse vaginal tract were evaluated using Student's *t* test or chi-square analysis. Statistical significance was accepted at a *P* value of $<0.05$.

## RESULTS

**GBS adheres to cells of the vaginal tract.** The ability of GBS to colonize the human vaginal tract was investigated *in vitro* using an established vaginal epithelial cell line, VK2/E6E7 (12). WT GBS strains A909, COHI, and NCTC10/84, representing the three most clinically dominant serotypes (Ia, III, and V, respectively), were incubated with VK2/E6E7 to determine the levels of both surface-adherent and intracellular bacteria using a previously described method for determination of GBS host cell adherence and invasion (9). Following a 30-min incubation and multiple wash steps to reduce nonspecific interactions, adherent bacteria were quantified from VK2/E6E7 lysates. All three GBS strains were able to adhere to the vaginal epithelial cells (Fig. 1A), with adherence values ranging from 40 to 60% of the initial input inoculum. Intracellular GBS was quantified from VK2/E6E7 lysates after a 2-h incubation followed by a 2-h exposure to antibiotics that kill only extracellular organisms. A relatively low level of intracellular bacteria was recovered, as only 0.05 to 1.25% of the initial inoculum was able to invade VK2/E6E7 cells (Fig. 1B). Thus, approximately 0.01 to 3% of the total VK2/E6E7-associated GBS, depending on the serotype, had invaded the intracellular compartment. This value is much lower than that reported previously for GBS invasion of other host cell types (9). Adherent GBS cells in association with vaginal epithelium were visualized by microscopy following multiple wash steps and Gram staining (Fig. 1C).

**Serine-rich repeat proteins and PilA contribute to adherence to vaginal epithelial cells.** The role of surface-associated proteins in GBS vaginal colonization was investigated using several isogenic mutant strains lacking Srr, PilA and PilB pilus components of PI-2a and surface-anchored lipoteichoic acid (LTA), which is diminished in the Δ*iagA* GBS strain (9). As shown in Fig. 1, both GBS WT strains NCTC10/84 and COH1 exhibited greater adherence (35 to 60%) to VK2/E6E7 cells than a Gram-positive non-human pathogen, *Bacillus thuringiensis* (1 to 2%) (Fig. 2A), or *Escherichia coli* (TOP10) (data

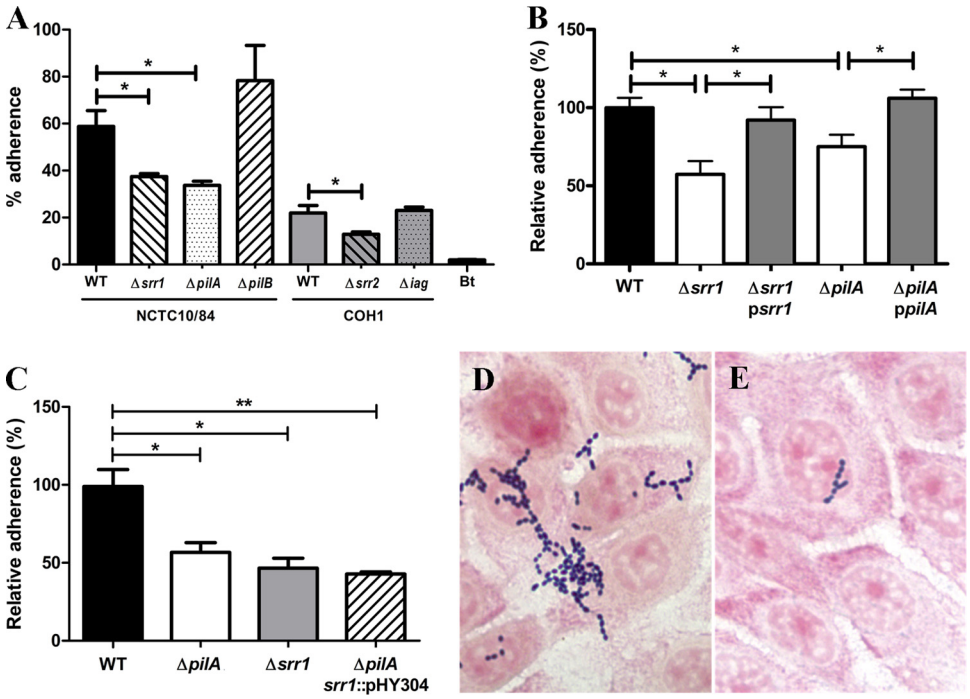


FIG. 2. (A) Adherence to VK2/E6E7 cells by WT GBS and Δ*pilA*, Δ*pilB*, and Δ*srr-1* mutant strains, derived from strain NCTC10/84, and Δ*srr-2* and Δ*iag* mutant strains, derived from strain COH1, as well as *Bacillus thuringiensis* (Bt) as a negative control. Data are expressed as the total adherent CFU recovered compared to the input inoculum (MOI of 1; ~1 × $10^5$ CFU). (B) Complementation of NCTC10/84 Δ*srr-1* and Δ*pilA* mutant strains was shown to restore adherence to vaginal epithelial cells; data are expressed as relative adherence compared to that of the parental strain. (C) Comparison of adherence levels of single mutants and the Δ*pilA srr-1*::pHY304 double mutant; data are expressed as relative adherence compared to that of the WT parental strain. All experiments were repeated at least three times in triplicate wells; data from a representative experiment are shown. Error bars indicate 95% confidence intervals of mean values from three wells. *, $P < 0.05$; **, $P < 0.005$. (D and E) Micrograph of Gram-stained VK2/E6E7 vaginal epithelial cells following inoculation with WT GBS NCTC10/84 (D) or the Δ*srr-1* mutant strain (E). Magnification, ×630.

not shown), which served as negative controls. Only the Δ*srr-1* and Δ*pilA* mutant strains exhibited significant decreases in VK2/E6E7 adherence compared to the parental strain (Fig. 2A), while the levels of invasion were not affected (data not shown). PilB or anchored LTA did not appear to contribute significantly to vaginal epithelial cell adherence (Fig. 2A). An isogenic mutant of the COH1 parental strain with a deletion in *srr-2*, the homologous gene allele to *srr-1*, was made and assayed in the *in vitro* model. The Δ*srr-2* mutant showed an ~30% decrease in cellular adherence compared to the WT (Fig. 2A), confirming that these adhesins in the *srr* gene family contribute similarly to vaginal attachment. Insertional *pilA* mutants of other GBS strains, NEM316 (serotype III) and 515 (serotype Ia), similarly exhibited decreased adherence to VK2/E6E7 cells (data not shown). Complementation of both the Δ*srr-1* and Δ*pilA* mutants with plasmid expression of *srr-1* and *pilA*, respectively, restored VK2/E6E7 adherence to WT levels (Fig. 2B). A mutant lacking both PilA and Srr-1 was constructed as described in Materials and Methods. This mutant (the Δ*pilA srr-1*::pHY304 mutant) exhibited decreased adherence levels that were similar to those of the Δ*srr-1* mutant (Fig. 2C), suggesting that while both of these surface factors contribute to vaginal attachment, the effect is not additive. Micrographs revealed vaginal cell-associated WT GBS (Fig. 2D), while very few adherent Srr-1-deficient organisms were observed (Fig. 2E).

**Srr-1 and PilA contribute to adherence to cervical epithelial cells.** GBS can often be isolated from the upper vaginal vault and cervical surfaces of human females (6). To examine upper vaginal tract colonization *in vitro*, we developed GBS adherence and invasion models of ecto- and endocervical cells using the respective Ect1/E6E7 and End1/E6E7 cell lines (12). All three GBS serotypes readily adhered to the cervical cell lines (Fig. 3A), and approximately 2 to 5% of the initial input inoculum was recovered inside the cell (Fig. 3B). Interestingly, the amount of the total cervical cell-associated GBS that was able to invade the intracellular compartment ranged from 5 to 30%, which was much higher than what we observed for vaginal epithelium. The role of Srr-1 and PilA in cellular adherence was then examined in the cervical epithelial cell lines. As was observed in the vaginal epithelium, the Δ*srr-1* and Δ*pilA* mutants demonstrated a significant decrease in adherence compared to that of the NCTC/1084 parental strain in both the Ect1/E6E7 and End1/E6E7 cell lines ($P < 0.001$) (Fig. 3C to G). Complementation of the mutant strains restored adherence to levels similar to that of the WT strain (Fig. 3C).

**Keratin 4 contributes to Srr-1-mediated attachment to vaginal epithelial cells.** Keratins are part of a family of fibrous proteins that contribute to structural elements of the cytoskeleton (13), and they have been shown to interact with a variety of bacterial pathogens (3, 46, 56). It has been reported previously that GBS binds to keratin 8 (48) and that Srr-1 specifi-

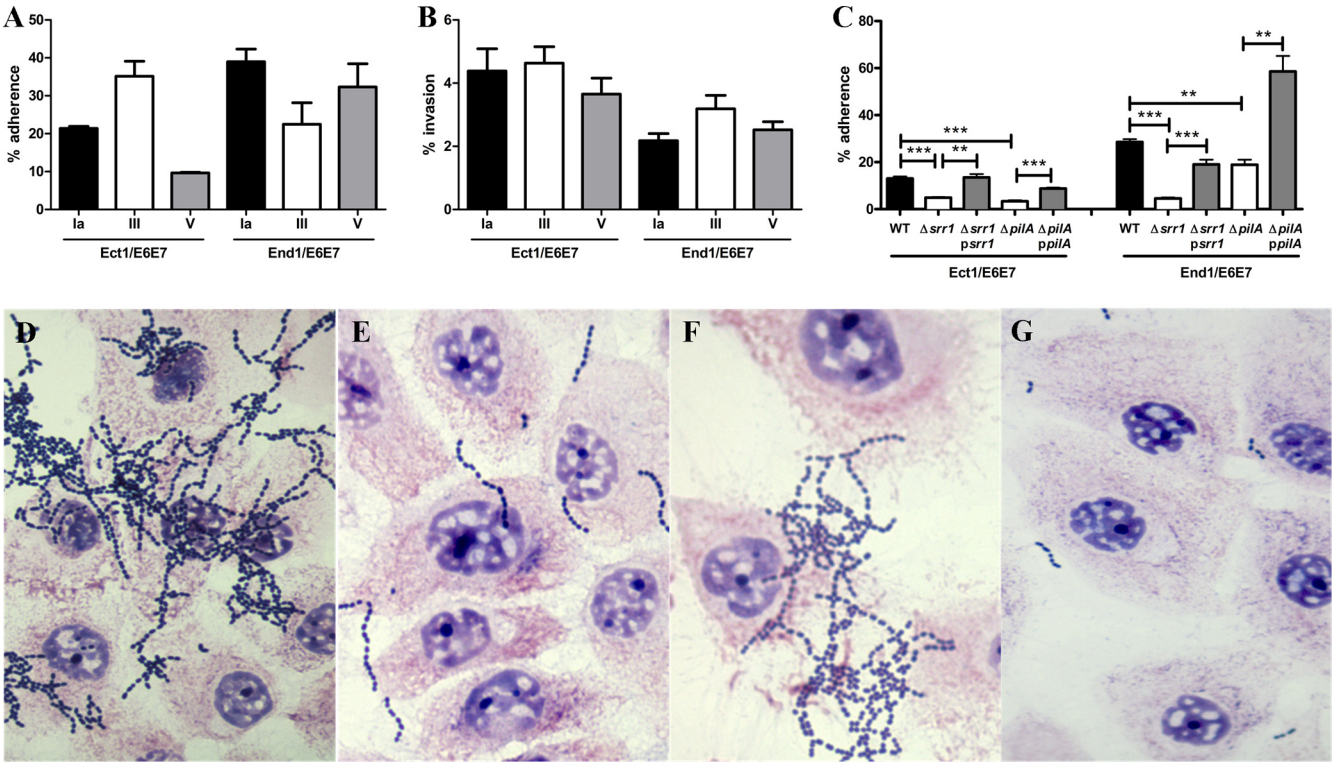


FIG. 3. GBS interaction with both ecto- and endocervical epithelia. (A and B) Adherence (A) and invasion (B) of Ect1/E6E7 and End1/E6E7 cells by representative GBS serotypes Ia, III, and V. Data are expressed as the total cell-associated (adherent) or intracellular CFU recovered compared to the input inoculum (MOI of 1; $\sim 1 \times 10^5$ CFU). (C) Complementation of NCTC10/84 Δ*srr-1* and Δ*pilA* mutant strains was shown to restore adherence to both ecto- and endocervical epithelia. All experiments were repeated at least three times in triplicate; data from a representative experiment are shown. Error bars indicate 95% confidence intervals of mean values from three wells. **, $P < 0.005$; ***, $P < 0.001$. (D to G) Micrograph of Gram-stained Ect1/E6E7 epithelial cells following inoculation with WT GBS NCTC10/84 (D) or the Δ*srr-1* mutant strain (E) and of End1/E6E7 epithelial cells following inoculation with WT GBS (F) or the Δ*srr-1* mutant strain (G). Magnification, ×630.

cally interacts with keratin 4 on the surface of host cells (41, 48). To assess whether keratin 4 may interact with GBS Srr-1 during vaginal colonization, VK2/E6E7 monolayers were incubated with monoclonal anti-keratin 4 antibody (KT4) or a mouse IgG1 isotype control, followed by the addition of WT or Δ*srr-1* mutant bacteria. We observed a 30% decrease in WT GBS adherence in the presence of the KT4 antibody compared to that with no treatment or isotype control treatment (Fig. 4A). As expected, the Δ*srr-1* mutant exhibited decreased adherence compared to that of the WT GBS strain. However, no change in Δ*srr-1* adherence was observed following preincubation with either KT4 or isotype control antibodies (Fig. 4A), suggesting that a Srr-1–keratin 4 interaction may mediate vaginal cell attachment.

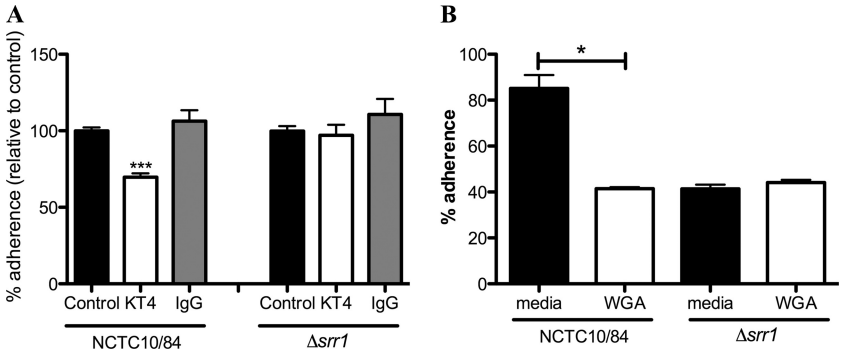


FIG. 4. Role of glycosylation and keratin binding in Srr1-mediated GBS cell attachment. (A) Adherence of GBS strains NCTC10/84 and the Δ*srr-1* mutant to VK2/E6E7 vaginal epithelial cells preincubated with anti-cytokeratin peptide 4 antibody (KT4) or an IgG1 isotope control. (B) Adherence of GBS strains NCTC10/84 and the Δ*srr-1* mutant to VK2/E6E7 vaginal epithelial cells preincubated with wheat germ agglutinin (WGA). All experiments were repeated at least two times in triplicate; data from a representative experiment are shown. Error bars indicate 95% confidence intervals of mean values from three wells. *, $P < 0.05$; ***, $P < 0.001$.

**Glycosylation of Srr-1 contributes to cell surface attachment.** Posttranslational modification of proteins through glycosylation involves the addition of specific carbohydrate moieties to the protein surface. Glycosylation is well characterized in eukaryotes but is a more recent finding in bacteria (31). Carbohydrate modifications are essential for protein function and have been associated with virulence in some bacterial species (31). Lectins bind carbohydrates with high specificity and can therefore be used to determine the exact nature of carbohydrate moieties present on certain proteins, as well as to interfere with functionality of the glycosylation (4, 53). We have previously shown an interaction between GBS Srr-1 and the *N*-acetylglucosamine (GlcNAc) binding lectin wheat germ agglutinin (WGA) (53), but it is unknown whether this and other carbohydrate moieties that may be found on Srr-1 are involved in promoting bacterium-host cell interactions. To assess this, various lectins, such as WGA, peanut agglutinin (PNA) (galactose/*N*-acetylgalactosamine binding), and concanavalin A (ConA) (mannose binding), were incubated with WT GBS and the Δ*srr-1* mutant and VK2/E6E7 cell monolayers. WGA reproducibly decreased WT GBS binding to vaginal epithelium (Fig. 4B) but did not alter the binding capacity of the Δ*srr-1* mutant (Fig. 4B). These results suggest that this carbohydrate modification contributes to GBS cellular attachment. PNA and ConA had no effect on GBS binding (data not shown), but we should note that it is unknown whether Srr proteins contain the specific carbohydrate moieties bound by these lectins.

**Srr-1 and PilA contribute to vaginal colonization *in vivo*.** Our *in vitro* data suggest that Srr-1 and PilA contribute to GBS cervicovaginal attachment. To test this ability *in vivo*, we developed a mouse model of GBS vaginal colonization using procedures similar to those described previously (5, 17, 24, 32, 40, 55, 63). Because bacterial colonization of the mouse vaginal vault is believed to be greater at estrus (5, 26), we synchronized the estral cycles of all mice using β-estradiol valerate. GBS ($1 \times 10^7$ CFU) was then introduced into the vaginal cavity, and on successive days the cervicovaginal vault of each mouse was swabbed with ultrafine swabs and recovered bacteria enumerated to monitor the bacterial load over time. Our results demonstrate that in the majority of animals, the Δ*srr-1* mutant was cleared from the vaginal environment more rapidly than the WT (Fig. 5A). By 4 days postinoculation only 30% of mice had Δ*srr-1* present in the vaginal tract, compared with 60% having quantifiable growth of the WT parental strain ($P = 0.028$). There was not a significant difference in the amounts of GBS recovered from mice infected with the Δ*pilA* mutant and from those infected with the WT strain (data not shown). To further assess the contribution of Srr-1 and PilA to GBS establishment in the vaginal tract, we examined vaginal colonization in a more sensitive bacterial competition model. Mice were inoculated simultaneously with equal amounts of both the WT and Δ*srr-1* or the WT and Δ*pilA* strains, and the presence of bacterial CFU was monitored at the indicated time point by antibiotic selection and PCR-based screening to distinguish between the WT and mutant strains. Consistently, more WT GBS than Δ*srr*-1 mutant (Fig. 5B) or Δ*pilA* mutant (Fig. 5C) GBS was recovered from the vaginal vault, suggesting that both Srr-1 and PilA contribute to GBS vaginal colonization.

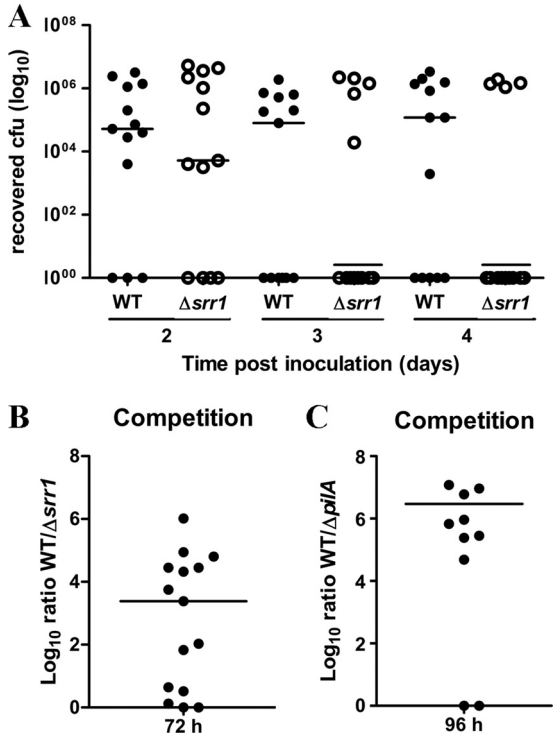


FIG. 5. Contribution of surface factors to GBS vaginal colonization *in vivo*. (A) Approximately $1 \times 10^7$ CFU of GBS NCTC10/84, WT, and Δ*srr-1* strains was inoculated into the vaginal vaults of 8-week-old female CD1 mice ($n = 13$). Persisting GBS colonies were enumerated at the indicated time points as described in Materials and Methods. (B and C) Competition assays where equal ratios of WT GBS and the Δ*srr-1* mutant (B) or WT GBS and the Δ*pilA* mutant (C) were introduced into the vaginal vaults of mice. CFU were enumerated at 72 to 96 h on THB agar and on THB agar supplemented with Cm to distinguish between WT and mutant bacteria. An overall ratio of 1 indicated equal persistence of WT and mutant GBS strains. All *in vivo* experiments were carried out in at least duplicate.

## DISCUSSION

GBS remains a significant neonatal pathogen despite widespread efforts to reduce infection. The vagina is recognized as a major reservoir of GBS, and vaginal colonization of pregnant women poses a significant threat to the fetus and newborn. To date very little is known regarding the molecular mechanisms that allow GBS to readily colonize the female cervicovaginal tract. We have developed both *in vitro* and *in vivo* models to investigate GBS surface factors that contribute to cellular association and persistence in the vaginal environment.

GBS is isolated from the vaginal tracts of women from all ethnic backgrounds and all stages of life (52, 58, 61). As an opportunistic pathogen, it rarely causes disease in healthy individuals unless host defense barriers are breached or the individual becomes immunocompromised. Capsular serotypes Ia, Ib, II, III, and V have been shown to account for upwards of 96% of neonatal and 88% of adult cases of GBS infection (34), indicating that the majority of strains are likely to share a suite of factors that assist in colonization and pathogenesis. In keeping with these findings, we determined that GBS strains of serotypes Ia, III, and V all readily adhered to both vaginal and cervical epithelia. A small percentage of these adherent organ-

isms were then able to subsequently invade the intracellular compartment of the host cell. The higher level of bacterial invasion observed in the cervical cell lines is particularly interesting and could suggest that cervical epithelium may provide an additional niche for GBS survival, but this warrants further investigation.

Pili have been only relatively recently described in Gram-positive streptococcal pathogens, including GBS (20). While pili are used for adhesion, motility, and conjugation in Gram-negative species, pili in Gram-positive bacteria are thought to be involved primarily in host cell attachment and biofilm formation (50). In GBS, pili have been shown to be essential for adherence and invasion of several human cell lines (10, 22, 23), but they have not previously been studied in relation to GBS vaginal colonization. Gram-positive pili, which are structurally distinct from the equivalent structures found in Gram-negative bacteria, are multimeric organelles made up of covalently linked proteins anchored to the bacterial cell wall. Our *in vitro* model demonstrated that the pilus tip adhesin, PilA, but not the backbone PilB protein, is involved in GBS attachment to vaginal and cervical epithelial cells. This is consistent with our previous findings for other cell lines, where we have shown a requirement of PilA for attachment to human brain microvascular endothelial cells while PilB plays a role in cellular invasion (22). The observed decrease in mouse vaginal colonization of an isogenic Δ*pilA* mutant while in competition with the WT strain confirmed our *in vitro* findings and suggests that GBS pili are likely to be involved in human vaginal colonization. The nature of this interaction as well as identification of a host cell receptor remains to be determined.

Streptococcal serine-rich repeat proteins have been repeatedly shown to contribute to host cell attachment. GspB and Hsa, which are Srr homologues found in *S. gordonii*, mediate binding of this bacterium to human platelets (47). Binding is specifically targeted to sialic acid-containing carbohydrate moieties on platelet membrane glycoprotein Ibα. The *S. pneumoniae* Srr homologue, PsrP, also functions as an adhesin by binding specifically to keratin 10 on the surface of lung epithelial cells (45). Keratins are intermediate filaments of epithelial cells and are essential for maintaining cell integrity. Mammalian keratins are divided into two groups, the acidic type I keratins and the basic type II keratins, and form heterodimers consisting of one keratin from each group (28). Keratin 4 and keratin 13 are paired in the epithelia of the esophagus, oral mucosa, and female genital tract (41), and homologues of these keratins also exist in the mouse (28). Our results suggesting that GBS Srr-1 may interact with keratin 4 on human vaginal epithelium are consistent with previous studies demonstrating that Srr-1 bound to keratin 4 on the surface of HEp-2 cells (41). As a blocking anti-keratin 4 antibody reduced WT GBS adherence by only 30%, it is likely that other bacterial and host factors contribute to overall GBS-vaginal cell interactions.

The genes for almost all of the streptococcal and staphylococcal serine-rich repeat homologues are chromosomally adjacent to genes involved in the glycosylation and transport of the proteins (47). Glycosylation of GBS Srr-1 was shown to be required for full virulence in a neonatal rat model of sepsis (27). Lectins have traditionally been used to identify specific carbohydrate moieties on glycosylated eukaryotic proteins. However, with increasing knowledge of glycosylation in prokaryotes, this technique has been successfully applied to bacterial glycobiology. Studies suggest that GspB of *S. gordonii* is glycosylated with carbohydrates, including *N*-acetylglucosamine (GlcNAc) and that these moieties are readily bound by wheat germ agglutinin (WGA) (4). We have previously shown that WGA binds to GBS Srr-1 (53), and we now have demonstrated that WGA can specifically block WT GBS binding to vaginal epithelial cells, although it is also possible that WGA may modulate receptors on the surface of the vaginal cells. This inhibition appears to be specific to the Srr-1 protein, as we observed no reduction in vaginal cell binding by the Δ*srr-1* mutant in the presence of WGA. The extent of Srr-1 glycosylation has yet to be determined but is likely to be important in determining the ability of GBS to bind host cells (27). Future studies determining the complete profile and nature of the carbohydrate modifications found on GBS Srr proteins should help dissect the complex multifactorial process of GBS binding to the human vaginal mucosa.

It remains difficult to truly replicate the complex interplay of factors influencing the human vaginal environment in a model system. However, murine models of bacterial persistence in the cervicovaginal tract have previously yielded useful results and allow a study of microbe-host interactions in a more representative environment. A similar model of vaginal colonization and infection has been used to demonstrate the susceptibility of female mice to multiple microbes, including *Neisseria gonorrhoeae* (17), *Chlamydia* (55), *Mycoplasma genitalium* (24), *Escherichia coli* (33), *Candida albicans* (40), herpes simplex virus 2 (32), *Trichomonas vaginalis* (11), and *Tritrichomonas foetus* (1). We have adapted this *in vivo* model to study GBS vaginal colonization in the presence of native microflora. Initial colonization experiments confirmed that GBS could be inoculated into the mouse vaginal vault and isolated at quantifiable levels for up to 8 to 12 days postinoculation (data not shown). This finding agrees with previous reports on murine models of vaginal infection for other pathogens (33, 63). Our observation that the majority of animals clear GBS within 8 to 12 days could be due to the host immune response or the normal murine microbiota, and both possibilities are under investigation. Some studies have found that if mice remain in estrus by continual hormone injection or if the normal microflora are eliminated by prior antibiotic treatment, microbial persistence can be extended. These are parameters that can be explored in our model for GBS in the future; however, our model still allows for the examination of GBS factors that contribute to the initial stages of GBS colonization and establishment in the vaginal tract.

One limitation of this mouse model is that the normal flora of the mouse vagina appears to differ from that of humans; however, previous studies have demonstrated that streptococci can be isolated from the mouse vagina (1, 25, 30). It is not known whether mice naturally harbor GBS specifically, but it will likely depend on the mouse strain, other flora present, and stage of estrus. We have demonstrated that both the Δ*pilA* and Δ*srr-1* mutants exhibited a reduced ability to persist in the vaginal tract compared to that of the WT strain. Only Srr-1 significantly promoted colonization in a single-strain challenge model, suggesting that Srr proteins may play a more prominent role in GBS vaginal colonization. We were not able to assess

complementation of the Δ*srr-1* mutant *in vivo*, as the plasmid vector containing the WT *srr-1* gene was not stable in the mouse vagina. This was surprising to us, as our competition experiments demonstrated that the presence of the *srr-l* gene provided a survival benefit in the mouse (Fig. 5B); thus, it would be expected that there would be a selective advantage to maintain the vector containing the WT *srr-1* gene. Our *in vitro* studies preformed previously (53) and presented here clearly demonstrate that the phenotype of the Δ*srr-1* mutant can be complemented, suggesting that the *srr-1* allele is expressed from the complementation vector. Thus, it is possible that during *in vivo* infection, Srr-1 protein expression in our complemented strain is not sufficient at an early time point to ensure plasmid stability. It is also likely that other unknown and/or known GBS adhesins/invasins, such as β-hemolysin/cytolysin (β-h/c) (8), fibrinogen binding protein (FbsA) (15), laminin binding protein (51), and the recently described HvgA (49), which has been shown to contribute to intestinal colonization, contribute to GBS-cervicovaginal tract interactions.

By combining *in vitro* and *in vivo* models, we have determined that GBS binds cells of the human cervicovaginal tract and is able to colonize the mouse vagina. A previously unidentified role in vaginal attachment has been attributed to GBS pili through the specific action of the tip adhesin PilA. Serine-rich repeat surface proteins also likely facilitate GBS attachment through binding to keratin 4 on the surface of host cells, an event mediated by the carbohydrate moieties that decorate the Srr-1 protein. Lastly, both Srr-1 and PilA contribute to vaginal survival in a mouse model of GBS colonization. Together these results represent a significant advance in our understanding of how GBS interacts in the vaginal environment and will assist in future studies on GBS vaginal colonization as well as the development of novel tactics for controlling GBS carriage in susceptible individuals.

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## REFERENCES

1. **Agnew, D. W., L. B. Corbeil, L. Munson, B. A. Byrne, and R. H. BonDurant.** 2008. A pregnant mouse model for bovine *Tritrichomonas foetus* infection. Vet. Pathol. **45:**849–864.
2. **Banerjee, A., et al.** 2011. Bacterial pili exploit integrin machinery to promote immune activation and efficient blood-brain barrier penetration. Nat. Commun. **2:**462.
3. **Batchelor, M., et al.** 2004. Involvement of the intermediate filament protein cytokeratin-18 in actin pedestal formation during EPEC infection. EMBO Rep. **5:**104–110.
4. **Bensing, B. A., B. W. Gibson, and P. M. Sullam.** 2004. The *Streptococcus gordonii* platelet binding protein GspB undergoes glycosylation independently of export. J. Bacteriol. **186:**638–645.
5. **Cheng, Q., D. Nelson, S. Zhu, and V. A. Fischetti.** 2005. Removal of group B streptococci colonizing the vagina and oropharynx of mice with a bacteriophage lytic enzyme. Antimicrob. Agents Chemother. **49:**111–117.
6. **Daimaru-Enoki, L. C., M. Morgan, W. S. Nichols, and N. S. Silverman.** 2005. First-trimester group B *Streptococcus* colonization of the cervix: a risk factor for maternal colonization at term? J. Reprod. Med. **50:**496–500.
7. **Desa, D. J., and C. L. Trevenen.** 1984. Intrauterine infections with group B beta-haemolytic streptococci. Br. J. Obstet. Gynaecol. **91:**237–239.
8. **Doran, K. S., J. C. Chang, V. M. Benoit, L. Eckmann, and V. Nizet.** 2002. Group B streptococcal beta-hemolysin/cytolysin promotes invasion of human lung epithelial cells and the release of interleukin-8. J. Infect. Dis. **185:**196–203.
9. **Doran, K. S., et al.** 2005. Blood-brain barrier invasion by group B *Streptococcus* depends upon proper cell-surface anchoring of lipoteichoic acid. J. Clin. Invest. **115:**2499–2507.
10. **Dramsi, S., et al.** 2006. Assembly and role of pili in group B streptococci. Mol. Microbiol. **60:**1401–1413.
11. **Escario, A., A. Gomez Barrio, B. Simons Diez, and J. A. Escario.** 2010. Immunohistochemical study of the vaginal inflammatory response in experimental trichomoniasis. Acta Trop. **114:**22–30.
12. **Fichorova, R. N., J. G. Rheinwald, and D. J. Anderson.** 1997. Generation of papillomavirus-immortalized cell lines from normal human ectocervical, endocervical, and vaginal epithelium that maintain expression of tissue-specific differentiation proteins. Biol. Reprod. **57:**847–855.
13. **Fuchs, E., and D. W. Cleveland.** 1998. A structural scaffolding of intermediate filaments in health and disease. Science **279:**514–519.
14. **Gaillot, O., C. Poyart, P. Berche, and P. Trieu-Cuot.** 1997. Molecular characterization and expression analysis of the superoxide dismutase gene from *Streptococcus agalactiae*. Gene **204:**213–218.
15. **Gutekunst, H., B. J. Eikmanns, and D. J. Reinscheid.** 2004. The novel fibrinogen-binding protein FbsB promotes *Streptococcus agalactiae* invasion into epithelial cells. Infect. Immun. **72:**3495–3504.
16. **Hammerschlag, M. R., et al.** 1977. Colonization with group B streptococci in girls under 16 years of age. Pediatrics **60:**473–476.
17. **Jerse, A. E.** 1999. Experimental gonococcal genital tract infection and opacity protein expression in estradiol-treated mice. Infect. Immun. **67:**5699–5708.
18. **Katz, V., and W. A. Bowes, Jr.** 1988. Perinatal group B streptococcal infections across intact amniotic membranes. J. Reprod. Med. **33:**445–449.
19. **Katz, V. L.** 1993. Management of group B streptococcal disease in pregnancy. Clin. Obstet. Gynecol. **36:**832–842.
20. **Lauer, P., et al.** 2005. Genome analysis reveals pili in group B *Streptococcus*. Science **309:**105.
21. **Madoff, L. C., J. L. Michel, and D. L. Kasper.** 1991. A monoclonal antibody identifies a protective C-protein alpha-antigen epitope in group B streptococci. Infect. Immun. **59:**204–210.
22. **Maisey, H. C., M. Hensler, V. Nizet, and K. S. Doran.** 2007. Group B streptococcal pilus proteins contribute to adherence to and invasion of brain microvascular endothelial cells. J. Bacteriol. **189:**1464–1467.
23. **Maisey, H. C., et al.** 2008. A group B streptococcal pilus protein promotes phagocyte resistance and systemic virulence. FASEB J. **22:**1715–1724.
24. **McGowin, C. L., R. A. Spagnuolo, and R. B. Pyles.** 2010. *Mycoplasma genitalium* rapidly disseminates to the upper reproductive tracts and knees of female mice following vaginal inoculation. Infect. Immun. **78:**726–736.
25. **McGrory, T., and G. E. Garber.** 1992. Mouse intravaginal infection with *Trichomonas vaginalis* and role of *Lactobacillus acidophilus* in sustaining infection. Infect. Immun. **60:**2375–2379.
26. **Medaglini, D., C. M. Rush, P. Sestini, and G. Pozzi.** 1997. Commensal bacteria as vectors for mucosal vaccines against sexually transmitted diseases: vaginal colonization with recombinant streptococci induces local and systemic antibodies in mice. Vaccine **15:**1330–1337.
27. **Mistou, M. Y., S. Dramsi, S. Brega, C. Poyart, and P. Trieu-Cuot.** 2009. Molecular dissection of the secA2 locus of group B *Streptococcus* reveals that glycosylation of the Srr1 LPXTG protein is required for full virulence. J. Bacteriol. **191:**4195–4206.
28. **Ness, S. L., et al.** 1998. Mouse keratin 4 is necessary for internal epithelial integrity. J. Biol. Chem. **273:**23904–23911.
29. **Nizet, V., et al.** 1997. Invasion of brain microvascular endothelial cells by group B streptococci. Infect. Immun. **65:**5074–5081.
30. **Noguchi, K., K. Tsukumi, and T. Urano.** 2003. Qualitative and quantitative differences in normal vaginal flora of conventionally reared mice, rats, hamsters, rabbits, and dogs. Comp. Med. **53:**404–412.
31. **Nothaft, H., and C. M. Szymanski.** 2010. Protein glycosylation in bacteria: sweeter than ever. Nat. Rev. Microbiol. **8:**765–778.
32. **Parr, M. B., et al.** 1994. A mouse model for studies of mucosal immunity to vaginal infection by herpes simplex virus type 2. Lab. Invest. **70:**369–380.
33. **Pascual, L., F. Ruiz, W. Giordano, and I. L. Barberis.** 2010. Vaginal colonization and activity of the probiotic bacterium *Lactobacillus fermentum* L23 in a murine model of vaginal tract infection. J. Med. Microbiol. **59:**360–364.
34. **Phares, C. R., et al.** 2008. Epidemiology of invasive group B streptococcal disease in the United States, 1999–2005. JAMA **299:**2056–2065.
35. **Poisson, D. M., M. L. Evrard, C. Freneaux, M. I. Vives, and L. Mesnard.** 2011. Evaluation of CHROMagar StrepB agar, an aerobic chromogenic medium for prepartum vaginal/rectal group B *Streptococcus* screening. J. Microbiol. Methods **84:**490–491.
36. **Pritzlaff, C. A., et al.** 2001. Genetic basis for the beta-haemolytic/cytolytic activity of group B *Streptococcus*. Mol. Microbiol. **39:**236–247.
37. **Ravel, J., et al.** 2011. Vaginal microbiome of reproductive-age women. Proc. Natl. Acad. Sci. U. S. A. **108**(Suppl. 1)**:**4680–4687.
38. **Regan, J. A., M. A. Klebanoff, and R. P. Nugent.** 1991. The epidemiology of group B streptococcal colonization in pregnancy. Vaginal Infections and Prematurity Study Group. Obstet. Gynecol. **77:**604–610.
39. **Rosini, R., et al.** 2006. Identification of novel genomic islands coding for antigenic pilus-like structures in *Streptococcus agalactiae*. Mol. Microbiol. **61:**126–141.

40. **Ryley, J. F., and S. McGregor.** 1986. Quantification of vaginal *Candida albicans* infections in rodents. J. Med. Vet. Mycol. **24:**455–460.
41. **Samen, U., B. J. Eikmanns, D. J. Reinscheid, and F. Borges.** 2007. The surface protein Srr-1 of *Streptococcus agalactiae* binds human keratin 4 and promotes adherence to epithelial HEp-2 cells. Infect. Immun. **75:**5405–5414.
42. **Schuchat, A., et al.** 1994. 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.
43. **Schuchat, A., et al.** 1990. Population-based risk factors for neonatal group B streptococcal disease: results of a cohort study in metropolitan Atlanta. J. Infect. Dis. **162:**672–677.
44. **Seifert, K. N., et al.** 2006. A unique serine-rich repeat protein (Srr-2) and novel surface antigen (epsilon) associated with a virulent lineage of serotype III *Streptococcus agalactiae*. Microbiology **152:**1029–1040.
45. **Shivshankar, P., C. Sanchez, L. F. Rose, and C. J. Orihuela.** 2009. The *Streptococcus pneumoniae* adhesin PsrP binds to keratin 10 on lung cells. Mol. Microbiol. **73:**663–679.
46. **Sojar, H. T., A. Sharma, and R. J. Genco.** 2002. Porphyromonas gingivalis fimbriae bind to cytokeratin of epithelial cells. Infect. Immun. **70:**96–101.
47. **Takamatsu, D., et al.** 2005. Binding of the *Streptococcus gordonii* surface glycoproteins GspB and Hsa to specific carbohydrate structures on platelet membrane glycoprotein Ibalpha. Mol. Microbiol. **58:**380–392.
48. **Tamura, G. S., and A. Nittayajarn.** 2000. Group B streptococci and other gram-positive cocci bind to cytokeratin 8. Infect. Immun. **68:**2129–2134.
49. **Tazi, A., et al.** 2010. The surface protein HvgA mediates group B streptococcus hypervirulence and meningeal tropism in neonates. J. Exp. Med. **207:**2313–2322.
50. **Telford, J. L., M. A. Barocchi, I. Margarit, R. Rappuoli, and G. Grandi.** 2006. Pili in gram-positive pathogens. Nat. Rev. Microbiol. **4:**509–519.
51. **Tenenbaum, T., et al.** 2007. *Streptococcus agalactiae* invasion of human brain microvascular endothelial cells is promoted by the laminin-binding protein Lmb. Microbes Infect. **9:**714–720.
52. **Tsui, M. H., et al.** 2009. Change in prevalence of group B *Streptococcus* maternal colonisation in Hong Kong. Hong Kong Med. J. **15:**414–419.
53. **van Sorge, N. M., et al.** 2009. The group B streptococcal serine-rich repeat 1 glycoprotein mediates penetration of the blood-brain barrier. J. Infect. Dis. **199:**1479–1487.
54. **Verani, J. R., L. McGee, and S. J. Schrag.** Prevention of perinatal group B streptococcal disease—revised guidelines from CDC, 2010. MMWR Recommend. Rep. **59:**1–36.
55. **Vonck, R. A., T. Darville, C. M. O'Connell, and A. E. Jerse.** 2011. Chlamydial infection increases gonococcal colonization in a novel murine coinfection model. Infect. Immun. **79:**1566–1577.
56. **Walsh, E. J., L. M. O'Brien, X. Liang, M. Hook, and T. J. Foster.** 2004. Clumping factor B, a fibrinogen-binding MSCRAMM (microbial surface components recognizing adhesive matrix molecules) adhesin of *Staphylococcus aureus*, also binds to the tail region of type I cytokeratin 10. J. Biol. Chem. **279:**50691–50699.
57. **Wessels, M. R., et al.** 1993. Stimulation of protective antibodies against type Ia and Ib group B streptococci by a type Ia polysaccharide-tetanus toxoid conjugate vaccine. Infect. Immun. **61:**4760–4766.
58. **Whitney, C. G., et al.** 2004. Group B streptococcal colonization in pregnant women. J. Matern. Fetal Neonatal Med. **15:**267–274.
59. **Wilkinson, H. W.** 1977. Nontypable group B streptococci isolated from human sources. J. Clin. Microbiol. **6:**183–184.
60. **Wilson, C. B., and W. M. Weaver.** 1985. Comparative susceptibility of group B streptococci and Staphylococcus aureus to killing by oxygen metabolites. J. Infect. Dis. **152:**323–329.
61. **Wood, E. G., and H. C. Dillon, Jr.** 1981. A prospective study of group B streptococcal bacteriuria in pregnancy. Am. J. Obstet. Gynecol. **140:**515–520.
62. **Zaleznik, D. F., et al.** 2000. Invasive disease due to group B *Streptococcus* in pregnant women and neonates from diverse population groups. Clin. Infect. Dis. **30:**276–281.
63. **Zarate, G., and M. E. Nader-Macias.** 2006. Influence of probiotic vaginal lactobacilli on *in vitro* adhesion of urogenital pathogens to vaginal epithelial cells. Lett. Appl. Microbiol. **43:**174–180.