**A 15-year single-center analysis of late-onset Group B Streptococcus infection correlating clinical severity with pathogen virulence determinants**.

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Summary: Post-EOD infant GBS disease is associated with high rates of ICU admission and meningitis without a consistent association with pathogen virulence, suggesting that host responses drive severity. We show that pathogen population structure was concordant globally and identify a novel MLST profile.

**Abstract**

**Background:** Group B Streptococcus (GBS) is a major cause of meningitis in infants, often prompting intensive care. The molecular factors leading to meningitis and other manifestations of severe infant disease are poorly understood. Furthermore, although Late-Onset Disease (LOD) and Very Late-Onset Disease (VLOD) appear similar clinically, the genomic differences between the GBS strains in LOD and VLOD have yet to be extensively described.

**Methods:** We characterized invasive GBS isolates from patients at Boston Children’s Hospital over 15 years using whole-genome sequencing. We compared isolate diversity with samples representing global contexts and examined vaccine coverage and antimicrobial resistance. Logistic regression and linear mixed models evaluated the relationships between clinical presentations or pathogen virulence factors and disease severity or age of onset, accounting for population structure and multiple testing.

**Results:** In the 87 patients studied, 44.3% needed ICU care, and 18.6% had meningitis. Among GBS, we identified 5 serotypes and 6 clonal complexes, with hypervirulent clones CC17/cpsIII and CC23/cpsIa being predominant. All isolates contained candidate polysaccharide conjugate and/or protein vaccine targets and were susceptible to penicillin and vancomycin, though 38% were resistant to erythromycin and 29% to clindamycin. ICU admission correlated with specific hematological abnormalities, but meningitis did not. No known virulence factors were associated with ICU or meningitis. LOD and VLOD did not differ bacteriologically, though the *PI-2A1* pilus gene was linked with a higher likelihood of VLOD.

**Conclusions:** The frequent ICU admissions and cases of meningitis observed in infant GBS invasive disease underscore its significant morbidity. However, the lack of molecular risk factors associated with severe disease highlights the need for further research into the host-pathogen interactions underlying infection. While the distinction between LOD and VLOD is widely accepted, the similar clinical presentation and genomic structure of the isolates in each group suggests that such a categorization is likely arbitrary.

# **Introduction**

*Streptococcus agalactiae* (Group B *Streptococcus* (GBS)), colonizes the vaginal or gastrointestinal tract asymptomatically but is capable of causing severe invasive infections (iGBS).[1] It is a common cause of sepsis and meningitis among neonates and infants globally.[1,2] In the United States, the Centers for Disease Control and Prevention (CDC) reports that GBS infections occur in approximately 0.4/1,000 infants under three months of age, with outcomes including mortality or long-term complications such as developmental delays, hearing loss, or cerebral palsy.[3,4]

Infant GBS disease is classified as early-onset disease (EOD) if diagnosed within the first seven days of life, late-onset disease (LOD) if diagnosed between seven days and three months, or very late-onset disease (VLOD), when occurring between three months and one year of age. EOD often results from vertical transmission and is effectively prevented through intrapartum antibiotic prophylaxis (IAP). Following IAP implementation in Europe and North America, cases of LOD are now more common than EOD.[5,6] This suggests that LOD might be acquired through different pathways besides vertical acquisition of commensal GBS with later translocation and invasion. [7] LOD and VLOD are considered clinically indistinguishable, but it is not known whether the bacterial isolates associated with each differ genomically, which couldaffect prevention and treatment strategies, including the coverage of vaccine candidates being developed for iGBS.[8,9]

The substantial clinical burden of GBS meningitis, especially its long-term neurodevelopmental impacts, is well-documented.[10–12] Serotype III strains of the hypervirulent clonal complex 17 (CC17) are the most common cause of meningitis and LOD, yet the molecular mechanisms that underlie neurotropism remain unclear.[4–6,10] Scarce data also limit the understanding of the molecular basis of other clinical features of acute infection in infants beyond meningitis, such as ICU admission rates.

In this study, we characterize the clinical and pathogen genomic characteristics of iGBS cases at Boston Children’s Hospital (BCH) over a 15-year period. Focusing on post-EOD disease, our dataset provides comprehensive clinical information on iGBS and the opportunity to examine any association between pathogen virulence determinants and clinical outcomes, including ICU admission and meningitis.

# **Methods**

Following ethics approval, we obtained 100 iGBS isolates (from blood or cerebrospinal fluid (CSF)) from patients at BCH collected between 2007 and 2021. The presence of bacteria in the blood indicated bacteremia, while meningitis was defined as positive GBS culture from the CSF and/or CSF pleocytosis. The associated demographic, antimicrobial sensitivity profile, laboratory, clinical information, and disposition (between Intensive Care Unit (ICU) and inpatient pediatric unit admissions) were extracted from hospital records.

*Whole-genome sequencing (WGS), antibiotic susceptibility testing (AST) and Genomic analysis*

Full descriptions of WGS, AST testing and Genomic analysis are detailed in supplementary material. Briefly, antimicrobial susceptibility was tested using disk diffusion on sheep blood Mueller-Hinton agar following CLSI guidelines, with antibiotics including penicillin, tetracycline, clindamycin, erythromycin, vancomycin and levofloxacin.[13] DNA from isolates was extracted and sequenced on the Illumina HiSeq 2500 platform to generate 100–150 bp paired-end reads.

Sequencing reads were quality-trimmed and screened for contamination. Isolates with <50% reads identified as Streptococcus agalactiae were excluded. Serotypes, sequence types (STs), clonal complexes (CCs), and key surface and resistance genes were identified using srst2 and GBS-Typer. Assemblies were quality-checked and screened for virulence factors. Core genome SNPs were used to infer a maximum-likelihood phylogeny using RAxML, rooted with a *S. pyogenes* outgroup. Duplicate isolates from blood and CSF were collapsed, resulting in 87 unique samples. Local LOD isolates (n=48) were placed in a global phylogenetic context using public datasets from the US, Netherlands, Malawi, Canada, and Ireland. Full code and pipelines are available at: <https://github.com/Leacavalli/BCH-GBS>. Clinical and Molecular Correlates of Severe Disease and post-EOD infection. The hematological data (WBC count, hemoglobin level, ANC, and platelet count, along with the occurrence of leukopenia, leukocytosis, and neutropenia) were examined across level of care (ICU vs. inpatient pediatric unit), and meningitis status. Logistic regression was employed to calculate odds ratios and 95% confidence intervals for each hematological indicator with Bonferroni correction for multiple testing. A genetic association analysis to identify molecular risk factors associated with clinical outcomes of interest including the age of disease onset, ICU admission, meningitis, and abnormal hematological parameters was conducted using a linear mixed model in pySeer to examine gene-phenotype associations incorporating patristic distances from the RAxML phylogeny to account for population structure.[14] Data analysis and visualizations were performed using R (V.4.3.1).[15]

Logistic regression with Bonferroni correction was similarly applied to compare the clinical presentation of LOD and VLOD, examining hematological data indices, ICU admission rates and the incidence of meningitis. We then examined potential differences in the strains of the GBS isolates present across age groups by comparing the distribution of clonal complexes and serotypes using Fisher’s test FDR-adjusted p-values. Finally, we used the linear mixed model in Pyseer to determine whether specific molecular risk factors associated with age of onset among infant beyond EOD. Age of onset was tested both as a binary outcome classified into LOD and VLOD, and as a continuous outcome, to account for the arbitrary nature of the 3-month LOD cutoff.

# **Results**

## *Clinical and Molecular Characteristics of iGBS*

There were 96 isolates from 87 patients with iGBS between 2007 and 2021. Meningitis was diagnosed in 18 infants (11 microbiologically confirmed + 7 with pleocytosis and concurrent bacteremia) and the remaining 69 had bacteremia without meningitis. The most common complaint and clinical sign at presentation were “fussiness” and fever, respectively. There were 70 infants with iGBS of which 2 were EOD, 48 LOD and 20 VLOD. (Figure [1](#_bookmark0), Table S1). The remaining 17 isolates were from 10 older children and 7 adolescents/adults. There were no deaths due to iGBS during hospitalization.

Throughout the study period, serotype III was the most prevalent in infants (n=45, 64.3%) followed by serotype Ia (n=15, 21.4%), which was also predominant among older children aged 1 to 18 years (n = 5, 50%) (Figure [1](#_bookmark0), Table S1). Six distinct Clonal Complexes (CCs) and 18 distinct Sequence Types (STs) among the iGBS isolates emerged with CC 17 (N=41, 47.1%) and ST-17 (n=33, 37.9%) being the most common as shown in Figure 1 and Table S2. Additionally, a new MLST profile not been reported in the pubMLST database was discovered: *adhP*(4) *pheS*(1) *atr*(4) *glnA*(4) *sdhA*(3) *glcK*(3) *tkt*(2). This new ST differed from ST-8 by one housekeeping gene (*glnA*), and from ST-10 by two housekeeping genes (*adhP* and *glnA*), classifying it within CC12 (Table S3). With one exception, each ST was associated with a single serotype, and each CC represented by a dominant serotype (Figure [2](#_bookmark1)). Finally, we found evidence of capsule switching in CC19 and CC12. In CC19, ST-19 and ST-335 were serotype III while ST-28 was serotype II. CC1/ST-1 was serotype V, and ST-459 was serotype IV. All CC12 isolates were serotype Ib, including the newly identified sequence type, with the exception of one ST-10/cps II isolate.

CCs differed between LOD and older children, LOD and adults, and VLOD and adults, (Figure [1](#_bookmark0)). There were also serotype differences between LOD and older children (p=0.002) and between LOD and adults (p=0.036). Although CC17/cps-III was more prevalent in LOD isolates (65%) compared with VLOD isolates (45%), CC23/cps-Ia showed the opposite trend (LOD: 15%, VLOD:30%), no significant difference was observed in the CC distribution between LOD and VLOD (p=0.095). Additionally, no clonal expansion or change in serotype distribution over time was detected (X-squared = 73.483, p-value = 0.365) (Figure [1](#_bookmark0)). The genetic diversity of LOD isolates was consistent with that observed in globally representative GBS populations encompassing various clonal complexes representative of distinct genotypes. (Figure 2B) There was no evidence of phylogeographic structure leading to localized transmission clusters by geographic location.

Two candidate GBS vaccines are currently under clinical evaluation.The hexavalent vaccine (GBS6 by Pfizer) would theoretically cover 100% of infant cases in our study (Figure [1](#_bookmark0), Table S4). The second vaccine, (by Minervax), is a dual-component GBS-NN and GBS-NN2which includes the N-terminal domains of αC/Rib and Alp1/Alp2/3. . [[10](#_bookmark12),11]. All infant isolates in our study contained genes encoding at least one Alp protein: *rib* (n=46, 66%), *alphaC* (n=7, 10%), *alp1* (n=15, 21%), and *alp23* (n=2, 3%) (Figure 2, Table S4). The *rib* gene was exclusively found in CC17 (n=40) and CC19 (n=6) isolates, *alphaC* in CC12 (n=7), *alp1* in CC23 (n=14) and CC459 (n=1), and *alp23* in CC23 (N=1) and CC1 (n=1) (Figure [2](#_bookmark1)).

All infant isolates encoded at least one of the three pilus islands: PI-1 (n=49, 70%), PI-2a (n=27, 39%) and PI-2b (n=38, 54%), as well as the *sip* gene (Table S4). In contrast, not all infant isolates carried genes encoding C5a peptidase (n=68, 97%), Lmb (n=69, 99%), and FbsB (n=55, 79%) (Table S4). The *FbsB* gene was exclusively present in hypervirulent clones CC17 (n=40, 100%) and CC23 (n=10, 100%) isolates, while absent from all other clonal complexes (Figure [2](#_bookmark1)).

## *Linking Phenotypic Antibiotic Resistance to Genotypic Markers*

All isolates were susceptible to penicillin and vancomycin. Thirty-three (38%) isolates exhibited resistance to erythromycin, in addition 23 (26%) were resistant to clindamycin, and 3 (3%) had intermediate resistance to clindamycin. Genes encoding macrolide resistance (*ermA*, *ermB*, *ermT*, and *mefA-msrD*) were identified in all erythromycin-resistant isolates and in 4 (7%) erythromycin-susceptible isolates. Genes linked to lincosamide resistance (*ermA*, *ermB*, and *ermT*) were found in all clindamycin-resistant isolates, 1 clindamycin-intermediate isolates, and 7 that were clindamycin-susceptible.

Tetracycline resistance was predicted in 78 isolates (90%) in our study from the presence of *tet-M* (n = 76, 87%), *tet-O* (n = 2, 2%), or both (n = 6, 7%). Aminoglycoside resistance was predicted in 4 isolates (5%) with the *aph(3’)-III* and *ant(6)* resistance genes, all belonging to CC17/ST-17 while *gyrA* or *parC* SNP variants associated with fluoroquinolone resistance were detected in three isolates. Isolates carrying the *aph(3’)III/ant(6)* genes only occurred from 2014 onwards. In contrast, the m*ef(A)-msr(D*) genes, were absent after 2013. Meanwhile, the frequency of *erm* genes, associated with resistance to both clindamycin and erythromycin, increased from 29% to 41%. (table S5)

## *Clinical and Molecular Correlates of Severe Infant Disease*

Among infant cases, 31 (44.3%) required ICU admission due to risks of clinical decompensation, mental status changes or escalating respiratory support. Leukocytosis, thrombocytosis and absolute neutrophil counts (ANC) were significantly correlated with reduced odds of ICU admission (Table 1). In line with this, neutropenia and leukopenia were associated with significantly higher odds of ICU admission (Table 1).Meningitis was documented in 18 out of 70 (25.7%) infants. No hematological parameters were significantly associated with meningitis after multiple testing corrections (Table S6). Infants and older patients exhibited similar clinical vitals with no significant differences in laboratory parameters on admission (Table 1). CC17 was significantly associated with infant infection (OR: 120.0, 95 % CI: 5.92 ;2434.31, p-value= 0.0018, Figure S3) and although *rib*, *pi2b*, *srr2*, *sip.3a*, *hgva*, and *fbsB* genes were more common in infant disease, these associations were not statistically significant after adjusting for population structure. Among infants, none of the virulence factors we evaluated were associated with ICU admission, meningitis, or abnormal hematological parameters (Tables S6–S11). Furthermore, ICU admission (χ2 = 6.58, p = 0.25), meningitis (χ2 = 3.91, p = 0.56), neutropenia (χ2 = 6.09, p = 0.30), leukopenia (χ2 = 8.72, p = 0.12), and leukocytosis (χ2 = 3.75, p = 0.59) were not significantly correlated with any clonal complex (Figure S3).

## *Bacteriological Similarities Between VLOD and LOD*

The pilus island *PI-2A1* gene was associated with reduced odds of LOD by 0.60 times compared with VLOD (95% CI: 0.42 - 0.85, p = 0.002). We also evaluated the association between *PI-2A1* and continuous age among infants to account for the 3-month cut-off for LOD, and found the effect remained similar (Table S13). No other virulence factors we investigated showed significant difference between LOD and VLOD isolates, reflecting the similarities in their strain distributions (Table S2).

The similarity of GBS strains causing LOD and VLOD suggests similar acquisition routes. Two cases of VLOD in twins we report offer insights into possible VLOD routes of acquisition. These infants presented with fever and seizures 107 days after birth. One twin was diagnosed with meningitis and admitted to the ICU while the other, without meningitis, remained in the inpatient unit. Isolates from both twins were identified as the CC23/cpsIa strain, with only 5 SNPs distinguishing them across their entire genomes. (Figure S1) Although their mother had negative GBS screening results, the genetic similarity of the isolates suggests a shared source of infection. While early intestinal colonization could explain the infections, simultaneous acquisition from an external source, such as community or enteral transmission, seems more plausible, similar to previously reported LOD twins.[16] There were no statistical differences between LOD and VLOD pertaining to odds of ICU admission, meningitis or other hematological indices (Table S6).

# **Discussion**

In this study characterizing post-EOD iGBS over a 15-year period, we observed significant acute morbidity among patients in our dataset, with 44.3% requiring ICU admission and 25.7% experiencing meningitis. While leukopenia, thrombocytopenia and neutropenia significantly increased the odds of ICU admission, we did not identify any bacterial virulence factors that correlated with the host-response. Unlike previous studies, our analysis did not show any association between the development of meningitis and the CC17/cpsIII strain, nor did we find any virulence factors attributable to meningitis. [5,10,17] This may reflect the limited power of our study due to a relatively small sample size. Alternatively, it might suggest the influence of host factors not captured in our study or complex host-pathogen interactions in determining disease severity and outcomes.

The clonal complexes and serotypes within our cohort reflect global trends, with hypervirulent clones CC17/cpsIII and CC23/cpsIa being predominant in cases of post-EOD iGBS. All invasive disease isolates encoded targets of both maternal vaccine candidates currently in development: GBS6 and GBS-NN2.[16,18] While these vaccines aim to prevent EOD and LOD, the observed potential coverage and the maternal antibodies they induce also suggest they could help prevent VLOD. [19,20] Moreover, the broad coverage of these vaccines in older pediatric and adult patients highlights their value for adult vaccination during outbreaks or in high-risk settings.[21] However, gaps in coverage due to non-vaccine types and non-typeable isolates underscore the need for post-vaccine GBS population monitoring to guide and inform future clinical and immunotherapeutic interventions.[22–24] Several other surface proteins have been identified as potential vaccine candidates due to their immunogenic nature observed in preclinical research.[25] The presence of genes encoding Sip and at least one pilus island in all our isolates supports their potential as targets in a universal GBS vaccine.[11] In contrast, genes for *C5a, Lmb, FbsB* and *srr1/2* proteins were absent from several of our isolates, which may limit their use as vaccine targets.

With regards to AST, there were no phenotypic or molecular determinants of non-susceptibility to penicillin and vancomycin, in agreement with other studies.[26] Similarly, no genetic markers for resistance were detected for other beta- lactams such as ampicillin or cefazolin, also recommended for AIP.[26] Conversely, approximately 29% and 40% of isolates were non-susceptible to clindamycin and erythromycin, respectively. The high rates of erythromycin resistance among iGBS isolates prompted its removal from U.S. AIP guidelines in 2010.[26] Although not statistically significant, the absence of *mef(A)/msr(D)* genetic markers and a rise in the prevalence of *erm* genes after 2013 could reflect changing antimicrobial pressures following the 2010 revision of AIP guidelines.[26] The ongoing presence of *ermA*, e*rmB*, and *ermT* genes, conferring resistance to both erythromycin and clindamycin, aligns with clindamycin’s continued use for GBS infections and other Gram-positive infections.[26–28]

Finally, VLOD cases, making up one-third of our cohort, showed no significant differences in clinical characteristics or strain diversity from LOD cases, with CC17/cpsIII prevailing in LOD and CC23/cpsIa in VLOD. Interestingly, the presence of the *PI-2A* variant 1 was associated with higher odds of VLOD. PI-2A stands out as particularly variable among the three GBS pilus islands.[29] The surface protein typing tool included PI-2A1 and PI-2A2 to maximize PI-2A coverage, rather than to signify distinct biological phenotypes.[29] Nonetheless, these subvariants are genomically distinct and may have significant biological differences. The conservation of PI-2A alleles indicates that genomic variations among subvariants likely do not affect functionality, suggesting PI-2A1 may not have unique virulence traits. Instead, the observation might result from antigenic variation due to immune selection pressures, such as prior exposure or waning maternal antibodies, which can increase susceptibility to different antigenic alleles as infants age—a pattern also seen in a related pilus locus in pneumococci.[30] Aside from these possible age-related changes in exposure and immune landscape, the similarities between LOD and VLOD cases suggest they are likely bacteriologically equivalent. This implies acquisition from the same niches, as supported by our case of VLOD twins pointing to simultaneous external acquisition—consistent with previous reports [7,16] emphasizing the arbitrary nature of the 3-month cut off and suggesting the VLOD cases might be included alongside LOD in studies of infant invasive disease. [5,6]

The single site of collection, absence of granular maternal colonization information and lack of information pertaining to disease onset are some of the limitations of this study. Although retrospective in nature and reliant on routinely collected data, our study provides valuable insights into the clinical presentation of iGBS in infants, clinical laboratory markers/predictors of severity, the distribution of host-responses driving clinical outcomes and the substantial overlap between LOD and VLOD in both clinical features and pathogen characteristics. In additions,the concordance in pathogen population structure with globally representative strains points to similar exposure to invasive serotypes across different geographies. Overall, our study generates further hypotheses regarding the complex host-bacterial interactions underlying iGBS while underscoring its clinical burden. It calls for broader investigations of the molecular risk factors linked to these outcomes, such as genome-wide association studies that require larger datasets, which are now becoming increasingly available.

**Legends to figures**

**Figure 1. Distribution of group B Streptococcus (GBS) clonal complexes (CCs) and**

**serotypes across age groups and over time.** The top panel displays the proportions of CCs across different age groups (left) and annually from 2007 to 2021 (right), while the bottom panel presents the corresponding distributions for serotypes. The age groups are defined as follows: early-onset disease (EOD) is diagnosed within 7 days of birth, late-onset disease (LOD) between 7 days and 3 months, very late-onset disease (VLOD) between 3 months and 1 year, older children range from 1 to 18 years, and adults are 18 years and older.

**Figure 2. Phylogenies of Boston Children’s Hospital (BCH) and Global Group B Streptococcus (GBS) isolates.** Panel A presents the phylogenetic tree of Group B Streptococcus (GBS) isolates from Boston Children’s Hospital (BCH), categorized by age groups: early-onset disease (EOD, diagnosed within 7 days of birth), late-onset disease (LOD, 7 days to 3 months), very late-onset disease (VLOD, 3 months to 1 year), older children (1 to 18 years), and adults (18 years and older). Various genes for virulence factors and surface proteins are detailed, including the Alpha-like proteins in blue (A :ALP1, B:ALP23, C:Alpha, D:RIB), pilus islands in green (E:PI-1, F:PI-2a1, G:PI-2a2, H: PI-2a, I: PI-2b), and other factors in orange such as the hypervirulence gene cluster A (J:HVGA), serine-rich repeat proteins (K:SRR1, L:SRR2), Sip (M), laminin-binding protein (N:lmb), C5a peptidase (O:scpB), hyaluronidase (P:hylB), and fibrinogen-binding protein (Q:fbsB). Panel B extends the context by situating BCH LOD isolates (in blue on heatmap 6) within a broader phylogenetic framework, including national and global LOD isolates. It features isolates from the USA (yellow on heatmap 6), gathered through the CDC’s ABCs program, and from other international sources such as Ireland, Malawi, Canada, and The Netherlands (orange on heatmap 6).

**Figure 3. GBS Phylogeny split by age of onset and disease severity.** (A) and (B) are phylogenies containing only infants and older patients with GBS infection, respectively. We observe that CC17-cpsIa is more common among infant cases. (C) and (D) are phylogenies containing only patients admitted to the ICU and those not, respectively. (E) and (F) are phylogenies containing only patients with and without meningitis, respectively.

**Table 1.** Hematological correlates of Clinical Outcomes

Infants (<1 years: EOD, LOD,VLOD) vs Older Patients (>1 year)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Older Patients (N=17) | Infants (N=70) | OR | (95% CI) | p-value |
| Hb Mean (SD) | 11.6 (2.5) | 10.9 (2.3) | 0.9 | (0.7-1.1) | 0.291 |
| WBC Mean (SD) | 10.5 (7.9) | 9.2 (6.4) | 1 | (0.9-1.1) | 0.476 |
| Leukocytosis N (%) | 6 (35.3) | 24 (35.3) | 1 | (0.3-3) | 1 |
| Leukopenia N (%) | 3 (17.6) | 16 (23.5) | 1.4 | (0.4-5.6) | 1 |
| Platelet Mean (SD) | 253.8 (205.2) | 334.8 (162.5) | 1 | (1-1) | 0.089 |
| ANC Mean (SD) | 8.2 (6.7) | 5.6 (4.9) | 0.9 | (0.8-1) | 0.085 |
| Neutropenia N (%) | 3 (18.8) | 13 (19.1) | 1 | (0.3-4.1) | 0.973 |
| ICU Admission among Infants | | | | | |
|  | Other (N=39) | ICU (N=31) | OR | (95% CI) | p-value |
| Hb Mean (SD) | 10.9 (1.8) | 10.9 (2.8) | 1 | (0.8-1.2) | 0.98 |
| WBC Mean (SD) | 11.7 (6.6) | 6.1 (5.4) | 0.8 | (0.8-0.9) | **0.001** |
| Leukocytosis N | 20 (52.6) | 4 (13.3) | 0.1 | (0-0.5) | **0.002** |
| Leukopenia N (%) | 4 (10.5) | 12 (40) | 5.7 | (1.6-20.1) | **0.002** |
| Platelet Mean (SD) | 390.5 (166.1) | 264.2 (128.8) | 1 | (1-1) | **0.003** |
| ANC Mean (SD) | 7.2 (4.6) | 3.5 (4.4) | 1 | (1-1) | **0.004** |
| Neutropenia N | 3 (7.9) | 10 (33.3) | 5.8 | (1.4-23.7) | 0.014 |
| Meningitis among Infants | | | | | |
|  | No (N=52) | Yes (N=18) | OR | (95% CI) | p-value |
| Hb Mean (SD) | 10.6 (2.1) | 11.8 (2.4) | 1.3 | (1-1.7) | 0.054 |
| WBC Mean (SD) | 10 (6.5) | 7 (5.6) | 0.9 | (0.8-1) | 0.089 |
| Leukocytosis N (%) | 20 (40) | 4 (22.2) | 0.4 | (0.1-1.5) | 0.183 |
| Leukopenia N (%) | 9 (18) | 7 (38.9) | 2.9 | (0.9-9.5) | 0.08 |
| Platelet Mean (SD) | 348.1 (168.6) | 298 (141.9) | 1 | (1-1) | 0.264 |
| ANC Mean (SD) | 6.2 (5.1) | 3.7 (3.7) | 1 | (1-1) | 0.065 |
| Neutropenia N (%) | 7 (14) | 6 (33.3) | 3.1 | (0.9-10.9) | 0.082 |

**References**

1. Shabayek S, Spellerberg B. Group B streptococcal colonization, molecular characteristics, and epidemiology. Front Microbiol 2018; 9:437.

2. Group B Streptococcus infection causes an estimated 150,000 preventable stillbirths and infant deaths every year. Available at: https://www.who.int/news/item/05-11-2017-group-b-streptococcus-infection-causes-an-estimated-150-000-preventable-stillbirths-and-infant-deaths-every-year. Accessed 6 May 2025.

3. Center for Disease Control, (CDC) P. ABCs Bact Facts Interactive Data Dashboard. 2024;

4. McGee L, Chochua S, Li Z, et al. Multistate, population-based distributions of candidate vaccine targets, clonal complexes, and resistance features of invasive group B streptococci within the United States, 2015–2017. Clinical Infectious Diseases 2021; 72:1004–1013.

5. Phares CR, Lynfield R, Farley MM, et al. Epidemiology of invasive group B streptococcal disease in the United States, 1999-2005. JAMA 2008; 299:2056–2065.

6. Nanduri SA, Petit S, Smelser C, et al. Epidemiology of invasive early-onset and late-onset group B streptococcal disease in the United States, 2006 to 2015: multistate laboratory and population-based surveillance. JAMA Pediatr 2019; 173:224–233.

7. Miselli F, Frabboni I, Di Martino M, et al. Transmission of Group B Streptococcus in late-onset neonatal disease: A narrative review of current evidence. Ther Adv Infect Dis 2022; 9:20499361221142732.

8. Cantey JB, Baldridge C, Jamison R, Shanley LA. Late and very late onset group B Streptococcus sepsis: one and the same? World Journal of Pediatrics 2014; 10:24–28.

9. Bartlett AW, Smith B, George CRR, et al. Epidemiology of late and very late onset group B streptococcal disease: fifteen-year experience from two Australian tertiary pediatric facilities. Pediatr Infect Dis J 2017; 36:20–24.

10. van Kassel MN, de Boer G, Teeri SAF, et al. Molecular epidemiology and mortality of group B streptococcal meningitis and infant sepsis in the Netherlands: a 30-year nationwide surveillance study. Lancet Microbe 2021; 2:e32–e40.

11. Bramugy J, Mucasse H, Massora S, et al. Short-and long-term outcomes of group B Streptococcus invasive disease in Mozambican children: results of a matched cohort and retrospective observational study and implications for future vaccine introduction. Clinical Infectious Diseases 2022; 74:S14–S23.

12. Paul P, Chandna J, Procter SR, et al. Neurodevelopmental and growth outcomes after invasive Group B Streptococcus in early infancy: A multi-country matched cohort study in South Africa, Mozambique, India, Kenya, and Argentina. EClinicalMedicine 2022; 47.

13. Weinstein MP, Lewis JS. The Clinical and Laboratory Standards Institute Subcommittee on Antimicrobial Susceptibility Testing: Background, Organization, Functions, and Processes. J Clin Microbiol 2020; 58:e01864-19. Available at: https://pmc.ncbi.nlm.nih.gov/articles/PMC7041576/. Accessed 15 May 2025.

14. Lees JA, Galardini M, Bentley SD, Weiser JN, Corander J. Pyseer: a comprehensive tool for microbial pangenome-wide association studies. Bioinformatics 2018; 34:4310–4312.

15. Team RC, others. R: A language and environment for statistical computing. Foundation for Statistical Computing, Vienna, Austria 2013;

16. Elling R, Hufnagel M, De Zoysa A, et al. Synchronous recurrence of group B streptococcal late-onset sepsis in twins. Pediatrics 2014; 133:e1388–e1391.

17. Chaguza C, Jamrozy D, Bijlsma MW, et al. Population genomics of Group B Streptococcus reveals the genetics of neonatal disease onset and meningeal invasion. Nat Commun 2022; 13:4215.

18. Absalon J, Segall N, Block SL, et al. Safety and immunogenicity of a novel hexavalent group B streptococcus conjugate vaccine in healthy, non-pregnant adults: a phase 1/2, randomised, placebo-controlled, observer-blinded, dose-escalation trial. Lancet Infect Dis 2021; 21:263–274.

19. Gonzalez-Miro M, Pawlowski A, Lehtonen J, et al. Safety and immunogenicity of the group B streptococcus vaccine AlpN in a placebo-controlled double-blind phase 1 trial. iScience 2023; 26.

20. Madhi SA, Anderson AS, Absalon J, et al. Potential for maternally administered vaccine for infant group B streptococcus. New England Journal of Medicine 2023; 389:215–227.

21. Watkins LKF, McGee L, Schrag SJ, et al. Epidemiology of invasive group B streptococcal infections among nonpregnant adults in the United States, 2008-2016. JAMA Intern Med 2019; 179:479–488.

22. Bianchi-Jassir F, Paul P, To K-N, et al. Systematic review of Group B Streptococcal capsular types, sequence types and surface proteins as potential vaccine candidates. Vaccine 2020; 38:6682–6694.

23. Croucher NJ, Finkelstein JA, Pelton SI, et al. Population genomics of post-vaccine changes in pneumococcal epidemiology. Nat Genet 2013; 45:656–663.

24. Bellais S, Six A, Fouet A, et al. Capsular switching in group B Streptococcus CC17 hypervirulent clone: a future challenge for polysaccharide vaccine development. J Infect Dis 2012; 206:1745–1752.

25. Dobrut A, Brzychczy-Włoch M. Immunogenic Proteins of Group B Streptococcus—Potential Antigens in Immunodiagnostic Assay for GBS Detection. Pathogens 2021; 11:43.

26. Verani JR, McGee L, Schrag SJ, others. Prevention of perinatal group B streptococcal disease: revised guidelines from CDC, 2010. 2010;

27. Pineles BL, Goodman KE, Pineles L, Harris AD. Appropriate antibiotic use for Group B streptococcus prophylaxis among penicillin-allergic patients in academic and nonacademic hospitals. In: Open Forum Infectious Diseases. 2022: ofac514.

28. Fay K, Onukwube J, Chochua S, et al. Patterns of antibiotic nonsusceptibility among invasive group A Streptococcus infections—United States, 2006–2017. Clinical Infectious Diseases 2021; 73:1957–1964.

29. Margarit I, Rinaudo CD, Galeotti CL, et al. Preventing bacterial infections with pilus-based vaccines: the group B streptococcus paradigm. J Infect Dis 2009; 199:108–115.

30. Regev-Yochay G, Hanage WP, Trzcinski K, et al. Re-emergence of the type 1 pilus among Streptococcus pneumoniae isolates in Massachusetts, USA. Vaccine 2010; 28:4842–4846.