Investigating the genetic factors that are associated with vertical transmission of Group B *Streptococcus* (GBS) in Nigeria

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Whole Genome Sequencing (WGS) tools can be used to investigate **transmission dynamics** of pathogens ***in vivo***

INTRODUCTION

- Group B *Streptococcus* (GBS) is an opportunistic bacterial pathogen that asymptomatically colonizes the genitourinary tracts of pregnant patients and can be transmitted to their neonate before or during childbirth[1]. Transmission can result in severe early- or late-onset neonatal disease, preterm births, or stillbirths.
- Intrapartum antibiotic prophylaxis (IAP) can be administered during labor and is recommended to prevent neonatal infection[1]. However, it is not implemented in Nigeria where vertical transmission is common (48.5%) and GBS-associated neonatal morbidities and mortalities are high (early-onset disease incidence of 2.0 cases per 1000 live births).
- GBS strains have a high phenotypic and genotypic diversity, which impact virulence and disease severity[2]. This variation is characterized through capsular serotyping, which categorizes strains into 10 categories based on capsule polysaccharides, or multilocus sequence typing, which categorizes strains based on genetic rather than phenotypic differences.
- Little is known about mutations that may arise in GBS following vertical transmission or whether certain genes or mutations are associated with enhanced transmission or invasive disease in newborns.

OBJECTIVES

We hypothesize that GBS vertical transmission and disease severity in newborns is impacted by the presence of specific virulence and resistance genes. To investigate this hypothesis, we will identify genes that are associated with enhanced transmission and invasive neonatal disease in Nigeria.

METHODS

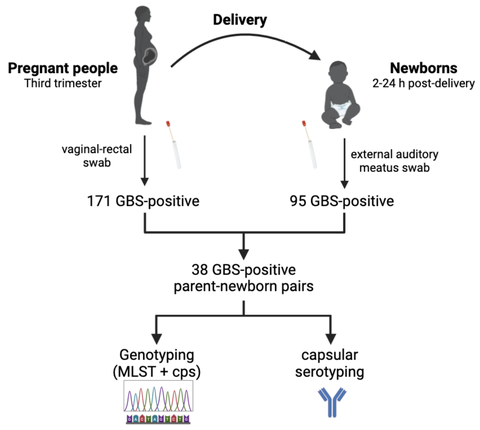

Figure 1. Schematic of GBS sampling method. GBS isolates were obtained from a previous cohort study of 500 pregnant people and their newborns in Nigeria[1]. Rectal and vaginal samples were collected from pregnant individuals in their third trimester of pregnancy, and samples were collected from the external auditory meatus of their newborns within 2-24 h after birth. None of the pregnant people received intrapartum antibiotic prophylaxis during labor. A total of 171 and 95 GBS-positive samples were obtained from parents and newborns, respectively. A subset of 38 GBS-positive parent-newborn pairs were selected from the cohort for whole genome sequencing (WGS) analysis.

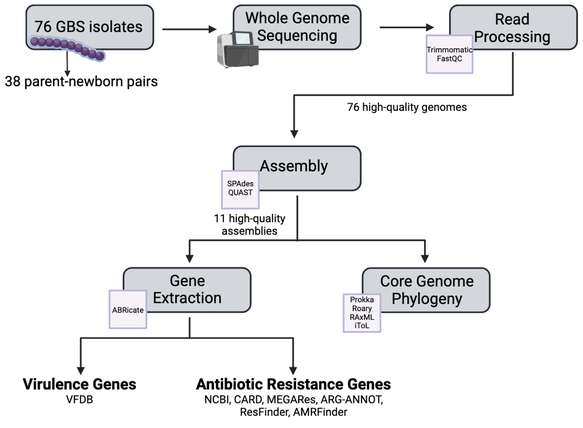

Figure 2. Methodology Schematic. 76 GBS isolates obtained from a prior cohort study of pregnant people and their newborns in Nigeria[1] were selected for WGS analysis. DNA was extracted and submitted for WGS on an Illumina MiSeq. The raw reads were trimmed (Trimmomatic) and checked for quality (FastQC). All 76 samples passed the quality control test and were assembled (SPAdes) before being assessed for quality (QUAST). Only 11 assemblies were considered high-quality to perform further analyses, including one parent-newborn pair. Gene extractions were performed with ABRicate[3] using multiple databases as references for antibiotic resistance genes and virulence genes. The high-quality assemblies were also annotated using a database of 120 GBS genomes from NCBI and the Prokka[4] pipeline, and a core-gene alignment was generated using the Roary[5] pipeline. Then, a maximum likelihood phylogeny tree was generated based on this core-gene alignment RAxML[6] and visualized through iToL.

RESULTS

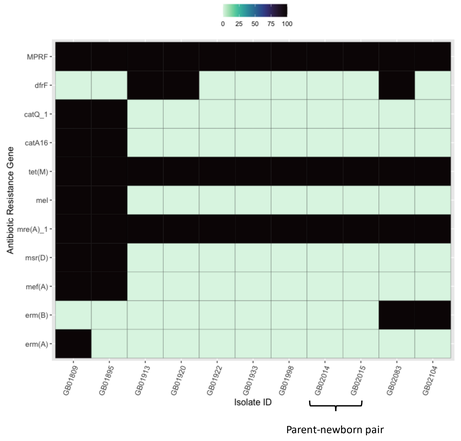

Figure 3. Occurrence of antibiotic resistance genes across sequenced isolates. Antibiotic resistance genes (ARGs) (*y*-axis) were extracted from 11 high-quality genomes (*x*-axis) using the ABRicate[3] pipeline and the following databases: NCBI, CARD, MEGARes, ARG-ANNOT, ResFinder, and AMRFinder. Genes conferring resistance to macrolide (*ermA, ermB, mefA, msrD, mreA, mel*), tetracycline (*tetM*), phenicol (*catA16, catQ*), and diaminopyrimidine (*dfrF*) antibiotics, as well as cationic peptides (*MPRF*) were identified. The percent nucleotide identities for each ARG is represented by the color gradient ranging from 0% (light green) to 100% (black), to indicate the absence or presence of each gene, respectively.

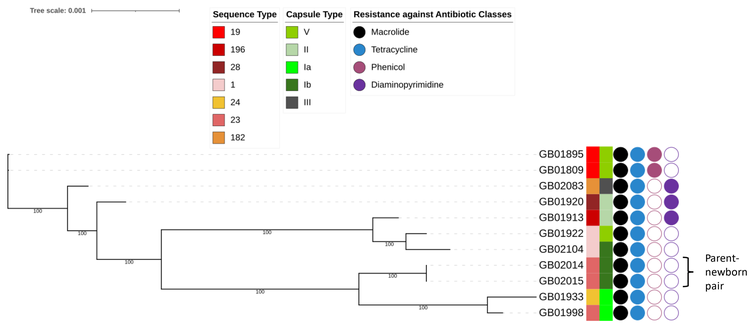

Figure 4. Core genome phylogeny tree for the sequenced isolates. The 11 high-quality genomes were annotated using the Prokka[4] pipeline and a maximum likelihood phylogeny was generated using RAxML[6] based on an alignment of core-genes using Roary[5]. Bootstrap values are shown at each node and branch lengths are shown to represent phylogenetic distance. The sequence type of each isolate is represented with squares in different shades of orange and red, while the capsule types are represented in different shades of green. The isolates that have antibiotic resistance genes that confer resistance to macrolide, tetracycline, phenicol, and diaminopyrimidine antibiotics are represented with filled circles (black, blue, mauve, and purple respectively).

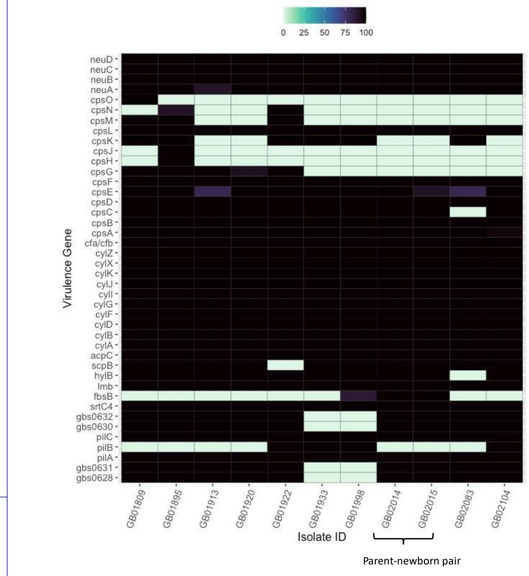

Figure 5. Occurrence of virulence genes across sequenced isolates. Virulence genes (VG) (*y*-axis) were extracted from high-quality assemblies of 11 isolates (*x*-axis), including one parent-newborn pair, using the VFDB database and the ABRicate pipeline[3]. The percent nucleotide identities for each VG is represented by the color gradient ranging from 0% (light green) to 100% (black), where genes with >80% nucleotide identity are considered present.

	Parent	Newborn
Paired	86	86
Unpaired	72	3
Invasive	1	1
Capsule Type Ia	18	11
Capsule Type Ib	12	8
Capsule Type II	38	19
Capsule Type III	18	13
Capsule Type IV	2	2
Capsule Type V	67	36
Total	**158**	**89**

Table 1. Characteristics of the GBS isolates recovered from pregnant people and their newborns in Nigeria. All of the GBS isolates recovered from the clinical study were characterized by capsule typing (serotyping). Samples that were duplicates (vaginal and rectal sample from the same pregnant person) or contaminated are not recorded (n = 19). Of the 247 isolates, 86 parent-newborn pairs were available for genome sequencing. One isolate that caused GBS disease in the newborn (invasive) and the paired parent isolate was included. Six capsule types were represented, with type V being the most common (n = 103), and type IV being the least common (n = 4).

CONCLUSIONS

1. Eleven different ARGs, providing resistance against four different antibiotic classes, were identified within the sequenced isolates (**Fig. 3**)
2. All eleven isolates had resistance genes against macrolide and tetracycline antibiotics (**Fig. 3, Fig. 4**)
3. Five out of the eleven isolates (45%) can be classified as multi-drug resistant (**Fig. 4**)
   1. Both ST-19 isolates are resistant to phenicol antibiotics
   2. Three isolates from varied ST and capsule types are resistant to diaminopyrimidine antibiotics
4. Within the sequenced isolates, 42 VGs were identified, including VGs for adherence to host cells, immune modulation, and exotoxin production (**Fig. 5**)
5. There were no differences in ARGs or VGs between the single parent-newborn pair (**Fig. 3, Fig. 4, Fig. 5**)

FUTURE DIRECTIONS

1. Sequence all 247 GBS genomes (**Table 1**) using a longer read length sequencing approach.
   1. Determine if ARGs, VGs or single nucleotide polymorphisms (SNPs) are acquired during transmission that may impact colonization or invasive disease in the newborns.
   2. Identify the virulence genes or other genetic markers necessary for transmission.

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REFERENCES

1. Medugu N, Iregbu KC, Parker RE, Plemmons J, Singh P, Audu LI, Efetie E, Dele Davies H, Manning SD. 2017. Group B streptococcal colonization and transmission dynamics in pregnant women and their newborns in Nigeria: implications for prevention strategies. Clin Microbiol Infect 23:673.e9-673.e16.
2. Luan SL, Granlund M, Sellin M, Lagergård T, Spratt BG, Norgren M. Multilocus sequence typing of Swedish invasive group B streptococcus isolates indicates a neonatally associated genetic lineage and capsule switching. 2005. *J Clin Microbiol*. 43(8):3727-3733. doi:10.1128/JCM.43.8.3727-3733.2005
3. https://github.com/tseemann/abricate
4. https://github.com/tseemann/prokka
5. Andrew J. Page, Carla A. Cummins, Martin Hunt, Vanessa K. Wong, Sandra Reuter, Matthew T. G. Holden, Maria Fookes, Daniel Falush, Jacqueline A. Keane, Julian Parkhill. 2015. Roary: Rapid large-scale prokaryote pan genome analysis. Bioinformatics, doi: http://dx.doi.org/10.1093/bioinformatics/btv421
6. Stamatakis A. 2014. RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. Bioinformatics 30 (9): 1312-1313.