Whole Genome Sequence based Capsular Typing and Antimicrobial Resistance Prediction of Group B Streptococcal Isolates from Colonized Pregnant Women in Nigeria

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Abstract

Background: *Streptococcus agalactiae* (Group B Streptococcus, GBS) is one of the major bacterial pathogens responsible for neonatal sepsis. Whole genome sequencing has, in recent years, emerged as a reliable tool for capsular typing and antimicrobial resistance prediction. This study characterized vaginal and rectal isolates of Group B Streptococcus obtained from pregnant women in Port Harcourt, Nigeria using a whole-genome sequence-based approach.

Results: Capsular types Ia, Ib, II, III, IV and V were detected among the 43 isolates sequenced. Twelve sequence types (STs) were identified, with ST19 (n=9, 27.3%) and ST486 (n=5, 15.2%) the most frequent among non-duplicated isolates. Of the alpha-like proteins (alp) identified, Alp1 was the most prevalent in 11 (33.3%) isolates. Macrolide and lincosamide resistance determinants were present in 15 (45.5%) isolates; *ermB* was detected in 1 (3%) and *ermTR* in 7 (21.2%) isolates. The *Inu* gene, was detected in 6 (18.2%) and *mef* was identified in 3 (9.1%) isolates. Resistance of GBS to erythromycin and clindamycin was found to be 30.3% and 24.2%, respectively. All isolates were resistant to tetracycline with only the *tetM* gene identified. Fluoroquinolone-resistance conferring substitutions in *gyrA* + *parC* were detected in 9 (27.3%) isolates and chloramphenicol resistance was predicted in 11 (33.3%).

Conclusion: The data available from the whole genome sequencing of these isolates offers a small but insightful description of common serotypes and resistance features within colonizing GBS in Nigeria.

Introduction

Streptococcus agalactiae, also known as Group B Streptococcus (GBS) is increasingly being recognized in Africa as one of the major pathogens responsible for neonatal sepsis¹⁻³. Vertical transmission from a rectovaginally colonized mother is the main predisposing factor for the development of early onset neonatal GBS sepsis^{4,5}. Some countries have policies regarding intrapartum antibiotic prophylaxis (IAP) to prevent early-onset disease (EOD) with resulting benefits^{6,7}. However, many low and middle income countries are yet to develop or implement such policies⁸. Whilst vaccine development is still underway^{9,10}, IAP remains one of the most effective tools for the prevention of EOD^{7,11}. The use of IAP may inadvertently result in the increase of antimicrobial resistance (AMR)⁸. For this reason, determination of antimicrobial resistance patterns is important, especially settings such as in Nigeria where there is high incidence of indiscriminate antibiotic use¹². Whole genome sequencing (WGS) has emerged as a reliable and cost-effective method for capsular typing and for accurate predictions of minimum inhibitory concentrations for antimicrobials in GBS^{13,14,15}.

This study analyses whole genome sequenced vaginal and rectal isolates of Group B Streptococcus obtained from previously described study in pregnant women in Port Harcourt, Nigeria¹⁶. Data from this study will add to existing evidence to guide vaccine efforts as well as contribute to local recommendations for intrapartum antibiotic prophylaxis.

Materials And Methods

Bacterial Isolates

A total of 46 isolates, obtained from separate vaginal and rectal samples of 185 pregnant women in their third trimester, collected retrospectively between March and July 2018 were used in this study. Samples were collected from women receiving antenatal care at the University of Port Harcourt Teaching Hospital in Port Harcourt Nigeria. Ethical approval was received from the hospital's ethics board and each participant signed a consent form prior to enrolment. Details of the study design and sample collection are described previously by Bob-Manuel et al¹⁶. Briefly, the rectovaginal samples were incubated overnight in LIM RambaQUICK™ and subcultured onto CHROMagar™ StrepB (CHROMagar, France). *Streptococcus agalactiae* were presumptively identified as small, mauve-coloured colonies on CHROMagar StrepB. Isolates were stored in tryptic soy broth containing 5% glycerol at -70°C. The isolates were shipped on Columbia agar with chocolate horse blood (Oxoid, UK) to the Streptococcus Laboratory, Centers for Disease Control and Prevention (CDC), for whole genome sequencing.

Whole genome sequencing and strain feature prediction

At CDC, GBS isolates were cultured on Trypticase soy agar supplemented with 5% sheep blood prior to DNA extraction from single colonies as previously described¹³. Following shearing of samples using a Covaris M220 ultrasonicator (Covaris, Inc, MA, USA) genomic libraries were constructed using sparQ DNA Library Kit (Quantabio, MA USA)^{13,14}. Short read sequences were generated on Illumina Miseq platform with MiSeq v2 500 cycle kit (Illumina Inc) and assembled de novo using VelvetOptimiser. Serotypes, multilocus sequence typing (MLST), antibiotic resistance determinants, and predicted minimum inhibitory concentrations (MICs) were determined using a previously validated GBS bioinformatics pipeline¹³. Isolates flagged for phenotypic testing (those with *Inu* gene and *ermTR* + *Inu*) were tested by Etest (Biomérieux USA), according to manufacturer's instructions. Additional strain features, including surface protein genes encoding the hypervirulent GBS adhesin (*hvgA*), serine-rich repeat (*srr*) proteins, alpha protein family (alpha, Rib, Alp2/3, Alp1), and pilus proteins predictive of pilus islands (designated here as PI1, PI2A, and PI2B) were extracted from genomic data (<https://github.com/BenJamesMetcalf>)¹⁴. Isolate identifiers, pipeline features, assembly metrics and NCBI genome accession numbers are listed in Supplementary Table 1.

Results

The proportion of vaginal/rectal samples positive for GBS was 16.8% (31/185) (sTable 1); 12 women had both vaginal and rectal swabs positive for GBS with 10 having identical serotypes and therefore counted only once in further analyses. Whole genome sequencing revealed 3 of the isolates to be non-GBS. Six of ten known capsular serotypes (Ia, Ib, II, III, IV and V) were detected within this sampling, with type V the most frequent (n = 11/33, 33.3%)(Table 1). Multilocus sequence typing (MLST) identified a total of 12 STs among the 33 strains with ST19 (n = 9, 27.3%) and ST486 (n = 5, 15.2%) most frequently occurring.

Eight (88.9%) of the ST19 sequence strains were serotype V isolates while serotype III accounted for 1 (11.1%) isolate. Only 1 serotype III isolate (3%) had the highly virulent ST17 clonal type.

Table 1
Distribution of sequence types (ST) by serotype for 33 non-duplicate GBS isolates

ST	Serotypes (%)					
	Ia	Ib	II	III	IV	V
1	-	-	-	-	-	1 (9.1)
2	-	-	-	-	2 (100.0)	-
8	-	1 (100)	-	-	-	-
17	-	-	-	1 (14.3)	-	-
19	-	-	-	1 (14.3)	-	8 (72.7)
23	1 (16.7)	-	-	-	-	-
24	4 (66.7))	-	-	-	-	-
26	-	-	-	-	-	1 (9.1)
28	-	-	2 (33.3)	-	-	1 (9.1)
182	-	-	-	3 (42.9)	-	-
486	1 (16.7)	-	4 (66.7)	-	-	-
1336	-	-	-	2 (28.6)	-	-
Total	6 (18.2)	1 (3.0)	6 (18.2)	7 (21.2)	2 (6.1)	11 (33.3)

The most predominant alpha-protein-like (alp) family present was Alp1 in 11 (33.3%) isolates, followed by Rib with 9 (27.3%), Alp2/3 with 6 (18.23%) isolates and 5 (15.2%) isolates were Alpha. Rib was detected in capsular types II, III and V; alp2/3 was detected in Ia, II and V; alp 1 in Ia, IV and V while the Alpha protein was detected in type Ia and Ib alone. Two isolates were negative for any of the alpha-protein-like family target predictions.

Serine-rich repeat proteins SSR1 and SSR2 were predicted in 29 (87.9%) and 1 (3%) of the isolates, while 3 (9.1%) were negative for both. Only one isolate demonstrated *hvgA*, a serotype III ST17 isolate as expected, the same isolate that was SSR2 positive. Isolates either had 1 or 2 pili; PI-1 + PI2A detected in 19 (57.6%), PI1 + PI2B in 8 (24.2%) and PI2A in 6 (18.1%) isolates.

Table 2 shows the antibiotic susceptibility as predicted by WGS and detection of specific resistance determinants. Macrolide and clindamycin resistance determinants were present in 15 (45.5%) of the

isolates with resistance rates to erythromycin and clindamycin of 30.3% and 24.2%, respectively. These determinants were present only in serotypes III, IV and V. The *MLS_B* genes which confer resistance to macrolides, lincosamides and streptogramin B, *ermB* was detected in 1 (3.0%) isolates and *ermTR* in 7 (21.2%) isolates; lincosamide nucleotidyltransferase determinant, *lnu*, was present in 6 (18.2%) and the determinant for macrolide efflux, *mef* was detected in 3 (9.1%) isolates. The 5 (15.2%) isolates with *lnu* alone and the one isolate with *ermTR* + *lnu* were susceptible to erythromycin + clindamycin and synergid, respectively, when tested phenotypically by Etest. Isolates with *mef/msrD* alone (2; 6.1%) were predicted as resistant to erythromycin but sensitive to clindamycin.

Table 2
Whole Genome Sequencing predicted antibiotic susceptibility for 33 non-duplicate GBS isolates

Antibiotic	Susceptible	Non-Susceptible
Ampicillin	33 (100)	-
Ceftriaxone	33 (100)	-
Chloramphenicol	25 (75.6)	8 (24.2)
Clindamycin	25 (75.6)	8 (24.2)
Ceftaroline	33 (100)	-
Daptomycin	33 (100)	-
Erythromycin	23 (69.7)	10 (30.3)
Levofloxacin	24 (72.7)	9 (27.3)
Meropenem	33 (100)	-
Linezolid	33 (100)	-
Penicillin	33 (100)	-
Synergid	33 (100)	-
Cefotaxime	33 (100)	-
Tetracycline	-	33 (100)
Vancomycin	33 (100)	-
* Quinupristin/Dalfopristin		

All isolates were resistant to tetracycline and the only gene detected conferring resistance was *tetM*. Fluoroquinolone-resistance conferring substitutions in both *gyrA* and *parC* were present in 9 (27.3%) isolates conferring full resistance phenotype, 8 (88.9%) of which were ST19 strains. Also, all of the isolates with fluoroquinolone resistance determinants were serotype V. The *aac6'-aph2*, which encodes

the bifunctional aminoglycoside-inactivating enzyme with 6'-acetyltransferase and 2"-phosphotransferase activities, were detected in 11 (33.3%) isolates and were exclusively in serotypes III, IV and V: 4 (36.4%), 1 (9.1%) and 6 (54.5%), respectively. CAT genes, encoding chloramphenicol acetyltransferases, were detected in 8 (24.2%) isolates, among serotypes III, IV and V.

Discussion

This study reports the molecular characterization of colonizing strains of Group B Streptococcus in the vagina and rectum of pregnant women. Serotype V was the most frequent serotype in this population representing 33.3% of the population.

Sequence types ST19, 486, 182, 24 and 2 accounted for 69.7% of the total isolates. This diversity is different from other reports that have reported ST19, ST23 and ST1 more commonly¹⁷. The highly virulent ST17 responsible for most invasive disease, especially late onset disease in neonates¹⁸, was rare in this study (3%) population which may suggest a better prognosis for neonates who may become colonized at birth in this environment. While ST182 was reported as a frequent clonal type by Medugu *et al* in another local study in Abuja, Nigeria², ST486 has not previously been documented as a common sequence type among GBS isolates in Nigeria.

Alp1 (Epsilon) (33.3%) was the most common of all the alpha-like proteins among the isolates in this study, similar to findings reported in Egypt (27%)¹⁹. Other studies have documented Rib as the most frequently detected Alp gene in other geographic regions like China and Iran (37.5% and 53% respectively)^{20,21}. The serine-rich repeat protein Srr-1 was identified in almost all isolates, however only a single isolate had the Srr-2 known to be associated with greater binding affinity and higher morbidity²². The same isolate was positive for the hypervirulent GBS adhesin (HvgA), another surface protein demonstrated to increase adhesion and virulence particularly in the ST17 lineage²³.

Similar to other studies from Nigeria^{2,24}, all isolates were determined to be susceptible to beta-lactam antibiotics including 3rd generation cephalosporins. This is quite reassuring, suggesting that penicillin and ampicillin remain as viable options for intrapartum antibiotic prophylaxis in this region despite concerns for antimicrobial resistance. For women allergic to penicillin, guidelines recommend clindamycin as an alternative option for prophylaxis²⁵. In this study however, clindamycin and erythromycin resistance were relatively high at 24.2% and 30.3%, respectively. Data from an earlier study in Nigeria reported erythromycin and clindamycin resistance rates of 6.5%²⁴ suggesting increased resistance rates within the country. However, all isolates were susceptible to vancomycin in this study, so patients with a high risk for penicillin anaphylaxis who are colonized with a clindamycin-resistant GBS strain, could receive vancomycin as the next best option²⁵.

Although other antibiotic classes such as fluoroquinolones, chloramphenicol, and tetracycline are not recommended for intrapartum prophylaxis, determining their susceptibility is important for tracking of resistance at the pneumococcal population level and as useful options for other invasive group B

streptococcal infections in other age groups. All isolates were predicted as tetracycline resistant which is consistent with several other reports documenting high levels of resistance to this antibiotic^{24,26,27}. Resistance to chloramphenicol and levofloxacin were also high at 25.6%. While these antibiotics may not be recommended for neonatal sepsis, (for which we screen mothers for GBS to prevent), they are useful options for other invasive group B streptococcal infections in other age groups.

The only tetracycline gene present in this population was *tetM*. Of all the tetracycline resistance determinants, the *tetM* has the most spread geographically among GBS^{24,26,27}. The presence of the double *gyrA* + *parC* mutants conferring full fluoroquinolone-resistance was high and were found predominantly in serotype V/ST-19 clone. The most frequently reported fluoroquinolone non-susceptible serotype/ST combinations are III/ST-19 and V/ST-19^{28,29} supporting the data from this study. Occurrence of either of these clonal complexes is likely a reflection of the circulating clones in those geographical areas in which they are found.

This study has demonstrated some salient characteristics of the prevalent colonizing strains of GBS in Nigeria, thus, contributing to the body of knowledge concerning GBS, as well as providing AMR data if IAP is considered in the future.

Declarations

Ethics approval and consent to participate: Ethical approval for the study was obtained from the University of Port Harcourt Teaching Hospital Ethical Committee (UPTH/ADM/90/S. II/VOL.XI/369). Written informed consent was obtained from each participant prior to study enrolment. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication: All authors contributed to study and manuscript development and consent to publication.

Availability of data and materials: Genome sequences has been uploaded to GenBank <https://www.ncbi.nlm.nih.gov/sra/PRJNA632041>. See sTable 1 for Biobank Accession numbers (SAMN18352391- SAMN18352411).

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Authors' contributions: Authors MB and LM conceived and designed the study. LM coordinated the whole genome sequencing and analyses. MB wrote most of the manuscript with major review and editing by LM. JI and MA helped with the data analysis. OO and KW contributed to critically reviewing the manuscript. All authors read and approved the final manuscript.

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