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Molecular Epidemiology of Invasive Group B *Streptococcus* in South Africa, 2019–2020

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Background. Group B *Streptococcus* (GBS) is a leading cause of neonatal meningitis and sepsis and an important cause of disease in adults. Capsular polysaccharide and protein-based GBS vaccines are currently under development.

Methods. Through national laboratory-based surveillance, invasive GBS isolates were collected from patients of all ages between 2019 and 2020. Phenotypic serotyping and antimicrobial susceptibility testing were conducted, followed by whole-genome sequencing for analysis of population structure and surface protein and resistance genes.

Results. In total, 1748 invasive GBS cases were reported. Of these, 661 isolates underwent characterization, with 658 yielding both phenotypic and genotypic results. Isolates (n = 658) belonged to 5 clonal complexes (CC1, CC8/10, CC17, CC19, and CC23) and 6 serotypes were detected: III (42.8%), Ia (27.9%), V (11.9%), II (8.4%), Ib (6.7%), and IV (2.3%). Phenotypically, only 1 isolate exhibited reduced penicillin susceptibility (minimum inhibitory concentration 0.25 µg/mL). Phenotypic resistance to erythromycin, clindamycin, and tetracycline was observed in 16.1%, 3.8%, and 91.5% of isolates, respectively. *ermTR* (34.9%) and *mefA/E* (30.1%) genes were most common among erythromycin-resistant isolates, while *ermB* predominated in clindamycin-resistant isolates (32.0%). *tetM* accounted for 95.8% of tetracycline resistance. All isolates carried at least 1 of the 3 pilus gene clusters, 1 of the 4 homologous alpha/Rib family determinants, and 98% harbored 1 of the serine-rich repeat protein genes. *hvgA* was found exclusively in CC17 isolates.

Conclusions. In our setting, β-lactam antibiotics remain appropriate for GBS treatment and polysaccharide and protein-based vaccines under development are expected to provide good coverage.

Keywords. *Streptococcus agalactiae*; vaccine targets; antibiotic resistance; molecular characterization; population structure.

Group B *Streptococcus* (GBS) is a leading cause of neonatal sepsis and meningitis [1]. In neonates and infants, GBS disease is classified as early-onset disease (EOD), which presents between days 0 and 6 of life [2], or late-onset disease (LOD), which presents between days 7 to 89 of life [3]. It also causes disease in adults, especially those with underlying medical conditions and older people [4].

Intrapartum antibiotic prophylaxis (IAP) is recommended for women at risk of transmitting GBS during the perinatal period [5]. This has dramatically decreased EOD in the United States [6]; however, it is logistically challenging to implement and maintain IAP in resource-constrained settings, such as South Africa. Most studies show GBS uniformly sensitive to penicillin, the recommended drug for IAP and GBS infections. However, several countries have reported cases of GBS with reduced penicillin susceptibility [7]. Additionally, resistance to second-line antibiotics such as clindamycin, erythromycin, and fluoroquinolones has increased in recent years in several countries [7]. Data on antibiotic susceptibility in South Africa are limited and surveillance is needed to understand the extent of antibiotic nonsusceptibility.

With the emergence of antimicrobial resistance, and lack of IAP in low-middle income countries, various serotype and protein-based vaccine formulations are in development. GBS

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is divided into 10 antigenically distinct serotypes: Ia, Ib, and II–IX [8]. A capsular polysaccharide vaccine targeting the 6 predominant serotypes (Ia, Ib, II, III, IV, and V) is currently undergoing a phase 1b/2 trial [9]. Protein-based candidates include pilus-based vaccines, the GBS-NN (which contains the N-terminus domains of AlphaC and Rib proteins), and the latch peptide vaccine (which targets serine-rich repeat proteins SRR1 and SRR2) [10–12].

In South Africa, the predominant GBS serotypes include Ia, Ib, II, III, and V, with serotypes Ia and III being most frequently identified in invasive disease isolates from both neonates and adults [13–17]. Surveillance efforts, including both active and passive surveillance, have provided valuable insights into the epidemiology of invasive GBS, particularly in neonates and infants [13–17]. However, additional data are needed across all age groups to better understand the serotype and surface protein distribution, especially in older adults. We aimed to characterize invasive GBS isolates from individuals of all ages in South Africa between 2019 and 2020, describing antimicrobial resistance, distribution of capsular serotypes and surface proteins, as well as genomic lineages.

METHODS

Bacterial Isolates and Phenotypic Characterization of GBS

As part of active, national, laboratory-based surveillance, GBS isolates and/or clinical specimens, along with demographic data from 2019–2020, were collected from all provinces in South Africa. These were forwarded to the national reference laboratory for characterization. An invasive GBS case was defined as an individual, of any age, with GBS isolated from a normally sterile site (eg, blood, cerebrospinal fluid) or nonsterile site specimen obtained from a patient with intrauterine sepsis.

At the reference laboratory, isolates were confirmed as GBS based on colony morphology characterized by small, translucent to opaque colonies, presence of narrow zones of β-hemolysis on 5% horse blood agar, and no hydrolysis on bile esculin agar. Isolates that did not morphologically appear as GBS were confirmed using matrix-assisted laser desorption/ionization-time of flight mass spectrometry.

Capsular serotyping was based on latex agglutination using the Immulex Group B *Streptococcus* antisera Ia, Ib, and II–IX (SSI Diagnostica) as previously described [18]. Isolates confirmed as GBS but nonreactive with all type-specific antisera were classified as serologically nontypeable.

Antimicrobial susceptibility testing was performed using both the disc diffusion method and broth microdilution method to determine minimum inhibitory concentrations (MICs) with Sensititre Streptococcus STP6F AST panels (ThermoFisher Scientific) for erythromycin, clindamycin, chloramphenicol, tetracycline, rifampicin, vancomycin, and cotrimoxazole. Results were interpreted as susceptible, intermediate, or resistant in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines [19].

DNA Extraction, Whole-Genome Sequencing, and Genome Assembly

DNA was extracted using the QIAamp DNA Mini kit (QIAGEN), with subsequent quantification using the Qubit 2.0 fluorometer (Invitrogen) and the Qubit dsDNA BR assay kit (Invitrogen). Library preparation was performed using the Illumina Nextera DNA Flex Library Preparation Kit, followed by whole-genome sequencing (WGS) on the Illumina NextSeq 550 System (Illumina) to generate 2×150-bp paired-end reads. We adapted the Centers for Disease Control and Prevention (CDC) GBS bioinformatics pipeline [20] (https://github.com/BenJamesMetcalf) to a containerized Nextflow version (https://github.com/shaze/GS) and used the pipeline for quality control and de novo assembly.

Bioinformatics Analysis

Serotype, multilocus sequence type (MLST), and antibiotic resistance determinants were determined from the genome data using the bioinformatics pipeline as previously described [20]. Resistance determinants included detection of mutations in the PBP genes (*pbp1a* and *pbp2x*) for penicillin nonsusceptibility, presence of macrolide and lincosamide resistance genes (*erm*B, *erm*A/TR, or *erm*C, and *mef*), fluoroquinolone resistance genes (*gyrA/gyrB* and *parC/parE*), and tetracycline resistance genes (*tet*). To ensure accuracy in comparisons between phenotypic results and genotypic predictions for serotype and antimicrobial resistance, tests were repeated when discrepancies were observed.

The pipeline was also used to detect the presence/absence of surface protein genes that encode the alpha protein family (*alpha, Rib, Alp1, Alp2/3*), hypervirulent GBS adhesion (*hvgA*), pilus islands (*PI-1, PI-2a, PI-2b*), and serine-rich repeat (SRR) proteins (*srr1* and *srr2*).

MLST relied upon the read mapping tool SRST2 and the PubMLST GBS database (https://pubmlst.org/organisms/streptococcus-agalactiae/) and determined sequence types by extracting the nucleotide sequences of the 7 MLST housekeeping genes (*adhP, pheS, atr, glnA, sdhA, glcK,* and *tkt*). Related sequence types (STs) were grouped into clonal complexes (CCs) using the global optimal eBURST (goeBURST) [21] algorithm based on sharing at least 6 of the 7 MLST loci, otherwise an ST was considered a singleton. The relationships between STs and different parameters were assessed using PHYLOViZ software version 2.0 (PHYLOViZ team) and illustrated by a minimum spanning tree [22].

Single-Nucleotide Polymorphism-Based Phylogenetic Analysis

Single-nucleotide polymorphism (SNP) detection was performed using the kSNP version 3 (http://ksnp.sourceforge.net/).

Table 1. Characteristics of Patients From Whom Group B *Streptococcus* Isolates Were Detected in South Africa, 2019–2020 (n = 1748)

Characteristic	Total Cases, n (%) (n = 1748)	Cases With Viable Isolates, n (%) (n = 661[a])	Cases Without Viable Isolates, n (%) (n = 1035[b])
Year			
2019	978 (55.9)	316 (47.8)	662 (60.9)
2020	770 (44.1)	345 (52.2)	425 (39.1)
Province			
Gauteng	752 (43.1)	244 (36.9)	508 (46.7)
Western Cape	344 (19.7)	203 (30.7)	141 (13.0)
KwaZulu-Natal	334 (19.1)	92 (13.9)	242 (22.3)
Eastern Cape	112 (6.4)	44 (6.7)	68 (6.3)
Limpopo	65 (3.7)	34 (5.1)	31 (2.9)
Mpumalanga	54 (3.1)	21 (3.2)	33 (3.0)
Free State	54 (3.1)	15 (2.3)	39 (3.6)
North West	17 (1.0)	5 (0.8)	12 (1.0)
Northern Cape	16 (0.9)	3 (0.5)	13 (1.2)
Sex			
Female	939 (53.7)	325 (49.2)	614 (56.5)
Male	751 (43.0)	317 (48.0)	434 (39.9)
Unknown	58 (3.3)	19 (2.9)	39 (3.6)
Age group			
0 to 6 d, EOD	590 (33.8)	250 (37.8)	340 (31.3)
7 to 89 d, LOD	420 (24.0)	183 (27.7)	237 (21.8)
3 mo to < 5 y	65 (3.7)	25 (3.8)	40 (3.7)
5 to <18 y	35 (18.6)	8 (1.2)	27 (2.5)
18 to 45 y	326 (12.6)	87 (13.2)	239 (22.0)
> 45 y	221 (5.2)	94 (14.2)	127 (11.7)
Unknown	91 (5.2)	14 (2.1)	77 (7.1)
Specimen type			
Blood culture	1230 (70.4)	533 (80.6)	697 (64.1)
Cerebrospinal fluid	194 (11.1)	71 (10.7)	123 (11.3)
Joint fluid	28 (1.6)	11 (1.7)	17 (1.6)
Pleural fluid	11 (0.6)	2 (0.3)	9 (0.8)
Other[c]	285 (16.3)	44 (6.7)	241 (22.2)

Abbreviations: EOD, early-onset disease; LOD, late-onset disease.

[a]Isolates sequenced and analyzed for this study.

[b]Includes audit cases and nonviable isolates.

[c]Includes specimen from tissue (lung, placenta, products of conception), bone, intrauterine death.

We selected samples that produced high-quality sequences (435/658, 66%) by filtering for those that had ≤ 150 contigs and N50 ≥ 30 000. We used a k-mer length of 19 and a requirement that 100% of the genomes have a nucleotide at a given SNP position in order for the SNP to be considered core. A maximum-likelihood (ML) phylogenetic tree was constructed using the generalized time reversible substitution model and gamma distribution in the RAxML tool using 100 bootstraps (https://github.com/stamatak/standard-RAxML). Ascertainment bias in SNP analysis was corrected using the Felsenstein correction. Phylogenetic trees were visualized with metadata using the Interactive Tree of Life online software (iTol version 6; https://itol.embl.de/).

Statistical Analyses

Distribution of serotypes with respect to age group and sex was assessed using the χ^2 test. A P value of <.05 was considered significant. All statistical analyses were performed in R version 4.1.2. Ages were grouped into EOD, LOD, 3 months to < 5 years, 5 to < 18 years, 18–45 years, and > 45 years.

Ethical Consideration

Ethics approval for GERMS-SA (protocol number, M1809107) and this substudy (M210476) was obtained from the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg, South Africa.

RESULTS

Patient Characteristics

During the study period, 1748 cases of invasive GBS were reported through GERMS-SA, with EOD (33.8%) and LOD (24.0%) most common, and 53.7% (939/1748) occurring in females (Table 1). Geographically, cases were widespread in all 9 provinces, with Gauteng contributing majority of the cases (43.1%).

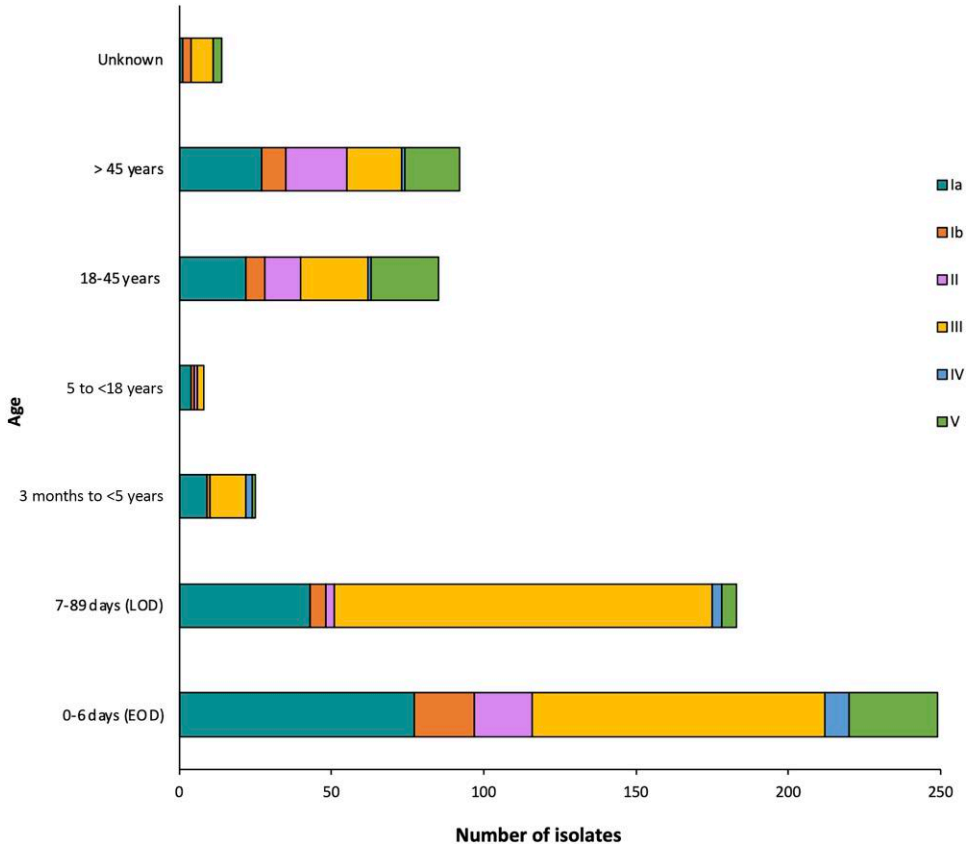

Figure 1. Distribution of invasive group B *Streptococcus* serotypes by age group in South Africa, 2019–2020 (n = 656). Abbreviations: EOD, early-onset disease; LOD, late-onset disease.

The reference laboratory received 714 (40.8%) samples, comprising 96.4% (688/714) isolates and 3.6% (26/714) specimens (culture negative cerebrospinal fluid [CSF], for polymerase chain reaction [PCR] confirmation). The remainder (1034/1748, 59.2%) were cases identified through a surveillance audit where no specimen/isolate was available for further characterization. Some patient demographic characteristics differed between cases with and without viable isolates (Table 1). Forty-nine percent (325/661) of cases with viable isolates were female, compared to 56.5% (614/1035) of cases with nonviable isolates. Among cases with viable isolates, 65.5% (433/661) were <3 months of age, compared to 53.1% (577/1035) of cases with nonviable isolates. Of the 688 isolates received, 661 (92.6%) were viable and underwent further characterization. Culture-negative CSFs were not further characterized. All 661 underwent both phenotypic characterization and WGS; however, 3 failed sequencing (Supplementary Figure 1). Here, we describe the 658 GBS cases for which WGS results were available.

Serotype Distribution

Phenotypic serotyping determined the serotype for 98.8% (650/658) of the isolates, and 8 (1.2%) were serologically nontypeable. In silico assignment predicted serotype for 99.8% (657/658) of isolates and 1 could not be determined. Excluding isolates lacking a serotype by either method (n = 9), the 2 methods demonstrated concordant results in 99.7% (647/649) of isolates (Supplementary Table 1). For subsequent analyses, isolates with discrepant results were excluded (n = 2). For isolates nontypeable by phenotypic methods (n = 8), the in silico serotype was used, and for the single isolate nontypeable by WGS, the phenotypic serotype was used.

Analysis of serotype distribution revealed that all isolates belonged to 1 of 6 predominant GBS serotypes (Ia, Ib, II, III, IV, and V), with serotype III being the most common, accounting for 42.8% (281/656) of all isolates (Figure 1). More than two-thirds (68.0%, 124/183) of LOD cases were caused by serotype III compared to 38.6% (95/249) of EOD cases ($P < .001$). In contrast, 30.9% (77/249) of EOD were due to serotype Ia compared to 23.5% (43/183) of LOD ($P < .001$).

Distribution of Surface Protein Determinants

Almost all isolates (99.8%, 657/658) harbored at least 1 of the 3 pilus determinants (Table 2). The most common pilus genes were a combination of PI-1 and PI-2b (41.1%, 270/657),

Table 2. Prediction of Surface Protein Determinants by Serotype in Invasive Group B *Streptococcus* Isolates Collected From Patients of All Age Groups in South Africa, 2019–2020 (n = 656[a])

Serotype	No. of Isolates (%)	*hvgA*	Serine-Rich Repeat Protein				Alp Protein Family				Pilus Island				
			srr1	*srr2*	*srr1: srr2*	Negative	*alp1*	*alp2/3*	*alpha*	*rib*	Negative	PI-2a	PI-2b	PI-1:PI-2a	PI-1:PI-2b	Negative
Ia	183 (27.9)	…	183 (52.7)	…	…	…	158 (96.9)	2 (4.9)	13 (12.0)	10 (2.9)	…	179 (81.7)	…	3 (1.9)	…	1 (100)
Ib	44 (6.7)	…	44 (12.7)	…	…	…	…	…	44 (40.7)	…	…	…	…	44 (27.8)	…	…
II	55 (8.4)	…	31 (8.9)	…	…	24 (75.0)	…	5 (12.2)	22 (20.4)	28 (8.2)	…	2 (0.9)	…	52 (32.9)	1 (0.4)	…
III	281 (42.8)	255 (97.0)[b]	12 (3.5)	261 (94.6)	1 (100)	7 (21.9)	…	1 (2.4)	…	280 (81.8)	…	…	4 (50.0)	19 (12.0)	258 (95.6)	…
IV	15 (2.3)	8 (3.0)[b]	…	15 (5.4)	…	…	…	…	7 (6.5)	8 (2.3)	…	…	4 (50.0)	…	11 (4.1)	…
V	78 (11.9)	…	77 (22.2)	…	…	1 (3.1)	5 (3.1)	33 (80.5)	22 (20.4)	17 (4.8)	2 (100)	38 (17.4)	…	40 (25.3)	…	…
Total	656[a]	263	347	276	1	32	163	41	109	341	2	219	8	158	270	1

Data are No. (%).

[a]Two isolates produced discordant serotype results and were excluded from this table.

[b]All belong to CC17.

followed by PI-2A (33.3%, 219/657), and a combination of PI-1 and PI-2a (24.4%, 160/657). The majority (95.6%, 258/270) of isolates that had a combination of PI-1 and PI-2b were serotype III, while 81.7% (179/219) of isolates with PI-2a were serotype Ia. Almost all isolates (99.7%, 656/658) harbored at least 1 of the 4 homologous genes of the alpha protein family determinants, with the *rib* protein determinant accounting for 60.0% (341/656) of the isolates. Most of *alp1*-positive isolates (96.9%, 158/163) were serotype Ia, and 80.0% (32/40) of *alp2/3* positive isolates were serotype V, while majority of *rib*-positive isolates (81.8%, 279/341) were serotype III. Serine-rich repeat glycoprotein determinants, *srr1* and *srr2,* were identified in 52.9% (348/658) and 42.1% (277/658) of isolates, respectively.

Antimicrobial Susceptibility Profiles and Resistance Mechanisms

All 658 isolates were tested individually for phenotypic susceptibility to each antibiotic, showing 100% susceptibility to linezolid and streptogramin. Penicillin susceptibility was observed in 99.8% (657/658) of the isolates, and 1 isolate (0.2%) showed reduced susceptibility to penicillin (MIC, 0.25 µg/mL). Prevalence of erythromycin and constitutive clindamycin resistance was 16.1% (106/658) and 3.8% (25/658), respectively. High tetracycline resistance rates were detected (91.5%, 625/658), while levofloxacin and vancomycin resistance were minimal (0.3% and 0.5% respectively). Frequencies of antimicrobial susceptibility patterns are given in Table 3 and Supplementary Table 3.

The isolates were further tested for the presence of resistance genes/mutations. The majority of the penicillin susceptible isolates possessed PBP2x types 1–5, and the single isolate with reduced susceptibility possessed PBP2x type 101. The most predominant genes in erythromycin-resistant isolates were *ermTR* (34.9%, 37/106) and *mefA/E* (29.2%, 31/106), while *ermB* was the most common in clindamycin-resistant isolates (8/25, 32.0%), followed by *ermTR* (4/25, 16.0%). Most (95.5%, 599/625) of the observed tetracycline resistance was associated with *tetM*. A combination of *gyrA*-S81L and *parC*-S87F mutations was observed in 1 of 2 levofloxacin-resistant isolates. Notably, within each antibiotic class, we observed a small number of phenotypically resistant isolates lacking resistance genes, alongside susceptible isolates harboring resistance genes, which were retested to eliminate the possibility of laboratory error, and the initial results were confirmed (Table 3).

MLST and SNP-Based Phylogenetic Analysis

MLST revealed 32 STs, 5 of which were novel. STs were grouped into 6 CCs (CC1 [37/658, 5.6%], CC8/10 [69/658, 10.5%], CC17 [272/658, 41.3%], CC19 [45/658, 6.8%], CC23 [188/658, 28.6%], and CC24 [37/658, 5.6%]) and 6 singletons (Supplementary Table 2 and Supplementary Figure 1). Maximum-likelihood phylogeny revealed 5 major clades

Table 3. Phenotypic Antibiotic Susceptibility of 658 Invasive Group B *Streptococcus* Isolates and Associated Resistance Mechanisms in Patients of All Age Groups in South Africa, 2019–2020

Antibiotic	Phenotype	Number of Isolates (%)	Genes/Mutations Detected, n (%)									
PBP2x type												
			1	2	4	5	39	101	178	New[a]	NF[b]	
Penicillin	S	657 (99.8)	164 (30.0)	203 (30.9)	3 (0.5)	200 (30.4)	61 (9.3)	0 (0.0)	8 (0.1)	4 (0.6)	17 (2.6)	
	RBLS	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	
Macrolide and lincosamide[c] resistance genes												
			ermB	*ermT*	*ermTR*	*mef*	*ermB:lsa*	*ermTR:mef*	*lsaC*	*lsa*	*lsaC:mef*	Neg
Erythromycin	S	537 (81.6)	1 (0.2)	1 (0.2)	1 (0.2)	1 (0.2)	1 (0.2)	1 (0.2)	–	–	1 (0.2)	530 (98.6)
	I	15 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.7)	0 (0.0)	–	–	–	14 (93.3)
	R	106 (16.1)	8 (7.5)	13 (12.3)	37 (34.9)	32 (30.1)	2 (1.9)	2 (1.9)	–	–	–	12 (11.3)
Clindamycin	S	620 (94.2)	1 (0.2)	13 (2.1)	34 (5.5)	–	1 (0.2)	2 (0.3)	0 (0.0)	2 (0.3)	0 (0.0)	566 (91.3)
	I	13 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	–	1 (7.7)	0 (0.0)	1 (7.7)	0 (0.0)	0 (0.0)	11 (84.6)
	R	25 (3.8)	8 (32.0)	1 (4.0)	4 (16.0)	–	2 (8.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.0)	9 (36.0)
Tetracycline resistance genes												
			tetM	*tetO*	*tetL:tetM*	*tetM:tetO*	*tet*	Neg				
Tetracycline	S	31 (4.7)	11 (35.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.2)	19 (61.3)				
	I	2 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2(100)				
	R	625 (91.5)	599 (95.8)	8 (1.3)	2 (0.3)	3 (0.5)	6 (1.0)	7 (1.1)				
Fluoroquinolone mutations												
			gyrA-S81L:*parC*-S83F	*parC*-D87N	*parC*-S83F	*parC*-S83Y	Neg					
Levofloxacin	S	654 (99.4)	1 (0.2)	1 (0.2)	8 (1.2)	2 (0.3)	642 (98.2)					
	I	2 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)					
	R	2 (0.3)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)					

Dashes represent cases where the gene is not applicable or does not confer resistance to the specific antibiotic.

Abbreviations: I, intermediate; Neg, negative; NF, not found (gene/mutation); R, resistance; RBLS, reduced β-lactam susceptibility; S, susceptible.

[a]New PBP2x types that are not yet assigned a number.

[b]Represents sequences where the PBP2x type could not be assigned to to insufficient sequence quality in the PBP2x locus.

[c]Clindamycin was tested only for constitutive resistance.

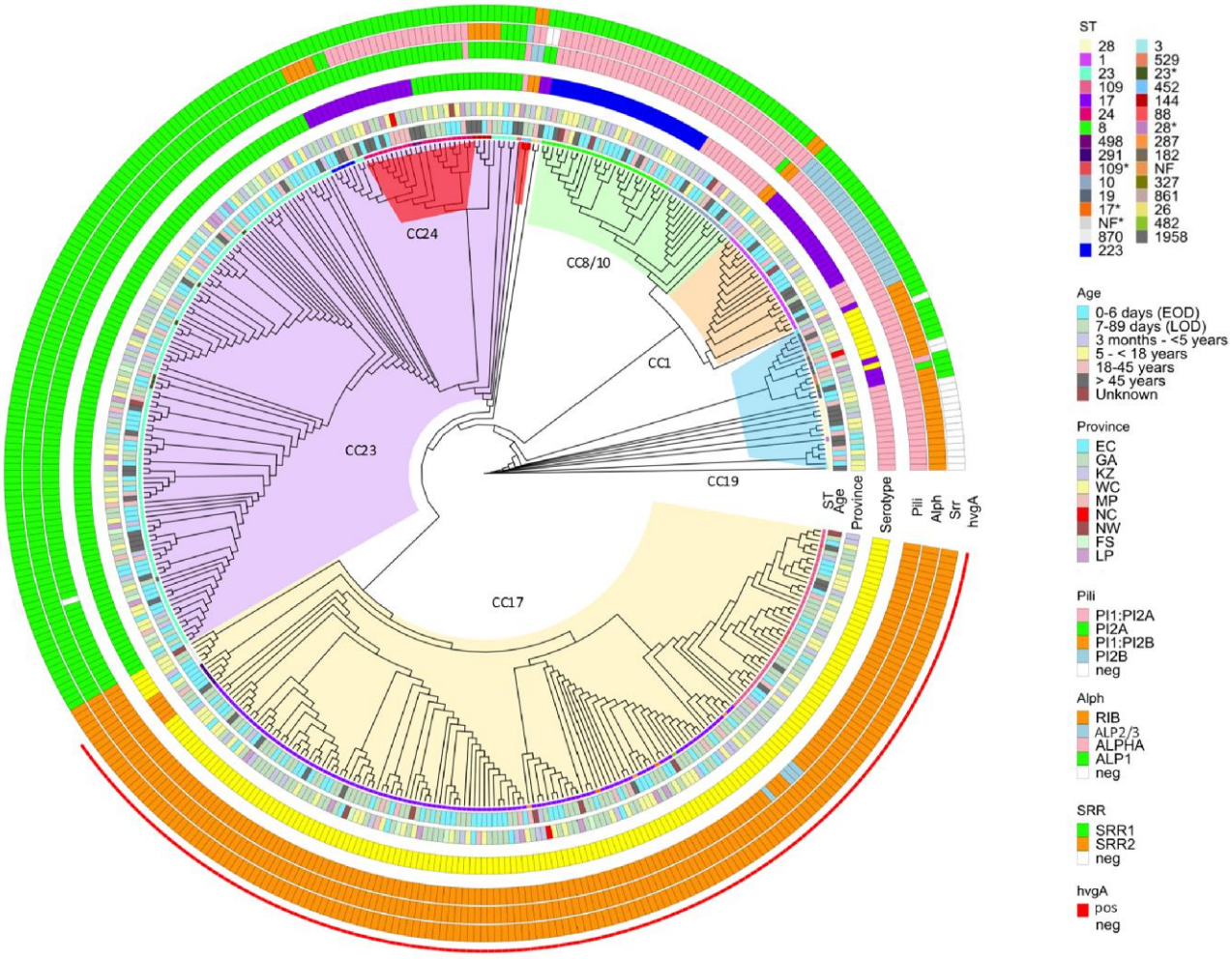

Figure 2. A core SNP-based maximum likelihood phylogeny of 435 invasive GBS isolates collected from South African patients of all age groups from 2019 to 2020. Branch highlights in the tree represent CCs and the inner thin strip correspond to STs. The subsequent strips (from inner to outer strip) indicate age, province, serotype (Ia–V), PI determinants, alpha protein family determinants, SRR protein determinants, and the hypervirulent GBS adhesion (hvgA) protein. Noncolored branches represent singletons STs that to do not fall within a specific CC. The tree only included isolates that produced high-quality sequences with ≤ 150 contigs and N50 ≥ 30 000. Asterisks represent STs that could not be confidently assigned due to insufficient quality during base calling on 1 of the 7 housekeeping genes; however, the overall genome quality was good. Abbreviations: CC, clonal complex; EC, Eastern Cape; EOD, early-onset disease; FS, Free State; GA, Gauteng; GBS, group B *Streptococcus*; KZ, KwaZulu-Natal; LOD, late-onset disease; LP, Limpopo; MP, Mpumalanga; NC, Northern Cape; neg, negative; NW, North West; PI, pilus island; pos, positive; SNP, single-nucleotide polymorphism; SRR, serine-rich repeat; ST, sequence type; WC, Western Cape.

(Figure 2 and Supplementary Figure 2). Four clades matched 4 CCs (CC1, CC8/10, CC17, and CC19); however, 2 CCs (CC23 and CC24) clustered into 1 clade (Figure 2). The 5 clades showed a varying level of intraclade serotype and protein-determinant diversity.

CC17 and CC23 were the largest and most homogenous. All but 5 CC17 isolates were serotype III, belonging to ST17 and a mix of its single locus variants. The 5 isolates were a cluster of ST291 isolates, which were all serotype IV. All isolates in the CC17 clade harbored *srr2* and the *rib* protein determinant, and almost all had PI-1:PI-2b. The *hvgA* was found exclusively in CC17.

CC23 and CC24 isolates were intermixed, forming a single clade. This clade (CC23/24) was primarily made up of serotype Ia and V isolates. Two serotype IV isolates formed a smaller subclade, and had a combination of surface protein determinants different from the rest of the subclades but matching that of CC17 isolates. The remaining CC23/24 subclades were mainly PI2A positive, and majority were *alp1* positive.

CC8/10 isolates formed 3 subclades: 1 comprising serotype Ib, another with serotype II, and a small subclade with serotype IV. All contained the *alpha* protein determinant. The serotype IV subclade possessed *srr2* and PI-1 + PI-2b, while all others had *srr1* and PI-1 + PI-2a (except 1 isolate with PI-2a).

CC1 had 2 subclades, 1 consisting of serotype II isolates and another with serotype V isolates. All isolates in this CC were *srr1* positive, possessed *alp2/3,* and a combination of PI-1 + PI-2a.

CC19 displayed 3 subclades, 1 with serotype II isolates, another with serotype III isolates, and a third with serotype V isolates. All but the cluster of serotype V isolates had the *rib* protein determinant and all had a combination of PI-1 + PI-2a. CC19 harbored all of the *srr*-negative isolates.

DISCUSSION

This study contributes data on serotype and antigen epidemiology of invasive GBS in South Africa, which may aid in prediction of the potential coverage and usefulness of GBS vaccines. Serotype and surface protein distributions suggest that vaccines currently under development would offer comprehensive coverage in South Africa.

Both polysaccharide or serotype-based conjugate and protein subunit GBS vaccines have reached phase 2 clinical trials [12]. We detected 6 serotypes (Ia, Ib, II, III, IV, and V), consistent with global data indicating that these are the predominant serotypes associated with invasive GBS disease [23]. The serotype distribution observed in this study is consistent with that reported in previous South African studies [23, 24]. Based on the observed serotype distribution, the polysaccharide conjugate vaccine (GBS6) that targets serotypes Ia, Ib, II, III, IV, and V has the potential to cover 100% of serotypes causing disease in South Africa. Additionally, the high concordance between phenotypic serotyping and WGS-based prediction of serotypes (99.7%) shows that in silico serotype assignment was sufficiently accurate for prediction of serotype.

Pilus islands are universally present in GBS and are therefore suitable targets for GBS vaccine development [25]. Our data are consistent with these findings, suggesting that a pilus-based vaccine that includes all 3 pilus islands would potentially target 99.8% of disease isolates. The GBS-NN vaccine has potential to protect against almost all GBS disease in South Africa because 99.7% of our isolates contained determinants for the highly homologous Alpha family proteins. This assumption is, however, uncertain, because even though these proteins are highly homologous, the N-terminal regions lack antigenic cross-reactivity and there is a lack of published data indicating cross-protection of GBS-NN against strains expressing Alp1 and Alp2/3 [25, 26]. The latch peptide vaccine has the capability to target 95.6% of disease-causing isolates in South Africa, which aligns with outcomes observed in preclinical models [27].

MLST has been the gold standard for characterization of many bacterial species; however, there has been some evidence of recombination in the housekeeping genes used by the MLST scheme in various organisms including *Staphylococcus aureus*, *Escherichia coli,* and *Salmonella enterica* [28–30]. Therefore, MLST-based phylogeny is not entirely free from noise created by recombination. Invasive GBS isolates in this study belonged to CC1, CC8/10, CC17, CC19, CC23, and CC24. The most common clonal complexes associated with disease globally are CC1, CC8/10, CC17, CC19, and CC23 [31]. Maximum likelihood phylogeny revealed 5 clades, 4 of which matched CC1, CC8/10, CC17, and CC19 perfectly. CC23 and CC24 isolates clustered within a single clade and this was similarly described in a UK study among isolates causing disease in adults between 2014 and 2015 [32]. In this UK study, CC24 (designated CC498/CC24) isolates were reassigned as CC23 based on the fact that CC498/CC24 isolates (assigned by MLST alone) were clustering within the CC23 clade in the core SNP-based phylogenetic tree. Therefore, core SNP may provide more resolution in terms of GBS population structure.

Although penicillin is still appropriate as first-line treatment for GBS infections and IAP and reduced susceptibility remains low, there are growing reports of isolates with reduced penicillin susceptibility from different countries including Japan, United States, Mozambique, Canada, European countries, Korea, and Hong Kong, with majority of the studies reporting <5% prevalence, with an exception of Japan which has shown an increase from 2.3% in 2005–2006 to 14.7% in 2012–2013 [7, 33–35]. Certain CCs, including CC17, CC1, and CC19, have been most commonly associated with reduced susceptibility in studies from various countries [36, 37]. Contrary to reports of increasing prevalence, we detected only 1 isolate with reduced penicillin susceptibility. Moreover, only 1 other study (2018) has reported this phenotype in South Africa (2 cases) [17], showing that it remains rare in the country, but monitoring is still required.

Resistance to second-line antibiotics, such as erythromycin and clindamycin, is rising globally [7], leading to changes in recommendations for adult second-line therapy to cephalosporins or vancomycin, as outlined in BMJ Best Practice Guidelines [38]. Erythromycin and clindamycin resistance in this study was lower than reported in other countries (<20% and <5%, respectively). Previous South African studies have also reported lower erythromycin and clindamycin resistance [17, 39]. Resistance rates of 55.2% and 43.9% were reported for erythromycin and clindamycin in invasive isolates in the United States in 2015–2017 [40]. Erythromycin resistance is high in China, with a prevalence of 74.1% observed in both colonizing and disease isolates [41, 42]. Vietnam has reported rates of 76.23% and 58.21%, respectively [43]. The relatively low levels of resistance observed in South Africa could be attributed to factors such as limited antibiotic use compared to other regions. Our findings support the reports that tetracycline resistance is high in GBS isolates with >95% resistance detected in this study. These results are comparable to what has been reported in France (95.5%) [44], Italy (93.3%) [45], and China (93.5%) [46]. Tetracycline is no longer used for treatment because its extensive use after its discovery led to high

resistance in several bacteria, including GBS [47]. In this study, tetracycline resistance was predominantly mediated by *tetM*. This is similar to what has been reported in the United States (92.8%) [40], France (95.0%) [44], and Italy (97.1%) [45].

We detected resistance-conferring genes/mutations in phenotypically susceptible isolates, which were not at the cusp of nonsusceptibility cutoff, in small fractions across the different antibiotic classes. This suggests that these genes were not expressed in these isolates. Both phenotypic testing and WGS for these samples was repeated from the same culture to eliminate laboratory error. Deekshit et al has shown different mechanisms that lead to silenced resistance genes in different bacterial populations and antibiotic classes [48]. Although reasons for the lack of expression were not examined, explanations may include mutations, absence of selection pressure (ie, absence of antibiotic), low expression levels of the gene, and the possibility of a weak, distant, or absent promoter. We also observed low numbers of phenotypically resistant isolates that lacked the targeted resistance mechanisms. This may be due to other resistance mechanisms not examined or not yet discovered. However, the most likely reason is that a resistance gene was present but not detected due to incomplete sequence coverage. Despite the few discrepancies, our results indicate that WGS sufficiently predicts the phenotypic serotype, as well as resistance in the majority of cases, demonstrating its reliability.

Our study had some limitations. First, not all of the cases reported to the GERMS-SA program had isolates received at the national reference laboratory for further analysis. In addition, viable isolates were only available for about half of the reported cases. Some characteristics of GBS cases with and without viable isolates differed, such as age group. As sequencing could only be performed for cases with viable isolates, our results may not be representative of all GBS cases in South Africa. Secondly, we were unable to identify all mechanisms of antibiotic resistance in the isolates. Thirdly, this study only included isolates collected over a 2-year period. This means that we were unable to monitor changes in reported GBS cases and surface protein distribution over time.

CONCLUSION

This study demonstrates that the hexavalent serotype-based GBS vaccine under development would offer 100% optimal coverage in South Africa. Additionally, we show that penicillin remains appropriate for treatment of GBS infections, with no noticeable increase in GBS with reduced penicillin nonsusceptibility. Moreover, we highlight the utility of WGS for in silico prediction of phenotypic characteristics.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Author contributions. B. N. contributed conceptualization, analysis, investigation, and wrote the original draft. S. W. and N. W. contributed conceptualization and data interpretation. B. M. performed bioinformatics analysis. S. H. performed bioinformatics pipeline set up. L. d. G. and D. M. performed investigations. S. M., F. M., and A. I. performed sequencing. S. C. performed bioinformatics analysis. A. v. G. performed data interpretation. All authors reviewed and edited the manuscript.

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Data availability. Data supporting this study are available at the European Nucleotide Archive at https://www.ebi.ac.uk/ena/browser/view/PRJEB78571.

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antimicrobial resistance and molecular characteristics of invasive early onset and late onset group B streptococcal disease amongst infants in South Africa, 2019–2020; International Conference on Group B *Streptococcus*, virtual, 20–21 July 2021, oral, Ntozini B, von Gottberg A, de Gouveia L, et al. Molecular characterization of invasive group B *Streptococcus* in South Africa, 2019–2020.

Potential conflicts of interest. S. M. has participated in an advisory board for GSK on meningococcal B vaccine use in South Africa, unrelated to this article. N. W. has received funding from Bill and Melinda Gates Foundation, World Health Organization, and Sanofi. A. v. G. is the chairperson of the National Advisory Group for Immunisation, South Africa. M. D. has received grant funding from the Bill and Melinda Gates Foundation. S. W. has received grant funding from the Bill and Melinda Gates Foundation, and the Centers for Diseases Control and Prevention. All other authors report no potential conflicts.

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