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Molecular Epidemiological Status of Group B Streptococcus in Ile Ife South Western Nigeria

**Akinlolu JT, Omololu-Aso J*,
Owolabi AT and
Omololu-Aso OO**

Obafemi Awolowo University, Ile-Ife, Nigeria

Abstract

Studies in some sub-Saharan African countries like Zimbabwe, Malawi, Kenya and Gambia revealed that Group B Streptococcus (GBS) is emerging as the main cause of neonatal sepsis and meningitis. However, in Nigeria, information on GBS disease prevalence remains sparse. We sourced to isolate GBS from the rectovaginal and neonatal samples that were obtained from a tertiary hospital in a populated area of Osun state and give an updated information on the antibiotic susceptibility patterns, using demographic and clinical parameters. 170 samples were collected from consenting mothers and neonate from June, 2016 to January 2017. Ninety-Eight (98) GBS isolates were recovered from vaginal, rectal of the pregnant woman at the point of labour and Umbilical cord of the neonate within 24 h of birth. Cultures for the isolation and identification of Group B Streptococcus (GBS) were carried out using the CDC recommended microbiological methods. The Kirby-Bauer disk-diffusion method was employed to determine antibiograms of GBS isolates in accordance with Clinical and Laboratory Standards institute (CLSI). The presence of resistant genes was examined using PCR. The prevalence rate of GBS maternal and neonatal colonization were 29.4% and 20.6% respectively while 4% of the colonized neonates had nosocomial GBS colonization. There was no significant association between GBS colonization status and age ($p>0.05$), parity ($p>0.05$), obstetric risk factors ($p>0.05$) and sex of neonate. One out of the 8 representatives of the multidrug resistant isolates harboured tetM gene while other resistant genes examined were negative in all MDR isolates. High prevalence of maternal and neonatal GBS colonization has been established among pregnant women and neonates in the study area. Nosocomial infection was implicated in GBS colonization among neonates. However further research is called for using larger sample size and multiple curve studies for adequate extrapolation into the general population.

Keywords: Group B streptococcus; Neonatal colonization; Pregnant women; Sepsis

*Corresponding author:

SJoseph Omololu-Aso

 omololu-aso@oauife.edu.ngObafemi Awolowo University, Ile-Ife,
Nigeria.**Tel:** +234 2348033770933

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Introduction

Group B Streptococcus (GBS) is the leading cause of neonatal infections in humans [1]. It is one of the main causes of infection in pregnant women with chorioamnionitis, endometritis, surgical wound infection as well as genital infections [2]. Approximately 10-30% of women of childbearing age carry GBS in the rectovaginal compartment [2]. A combination of phenotypic classification and molecular typing has been successfully used in epidemiological investigations of GBS to study clonal lineages associated with colonization or invasive disease [3,4]. A diverse range of molecular techniques have been applied to

determine the population structure of GBS, including pulse field gel electrophoresis (PFGE), restriction digest pattern (RDP) and multilocus sequence typing (MLST) [5-7].

Multilocus sequence typing has several advantages over restriction digest based molecular typing techniques in that it uses standardized protocols, and provides specific data on single nucleotide changes rather than crude estimates of approximate fragment length. Furthermore, the MLST data from different laboratories can be stored and compared on an open access online database, and hence it has become the preferred method to compare the genetic relatedness of GBS isolates [8].

Multilocus sequence typing has been successfully used to characterize the clonal diversity of a number of human bacterial pathogens including *Neisseria meningitidis* and *S. pneumoniae* [9,10]. Multilocus sequence typing is based on the DNA sequencing of seven housekeeping GBS genes, which include alcohol dehydrogenase (*adhP*), phenylalanyl tRNA synthetase (*PheS*), amino acid transporter (*atr*), glutamine synthetase (*glnA*), serine dehydratase (*sdhA*), glucose kinase (*glcK*) and transketolase (*tkt*) [7]. Based on the Multi-Locus Sequence Typing (MLST) data, a number of different sequence types (STs) have been described in maternal and neonatal colonizing GBS isolates. Despite variability in the proportionality of clonal lineages, molecular characterization of GBS colonizing isolates has shown a limited number of major sequence types as associated with colonization regardless of geographical and epidemiological distinction [11,12]. There are four major MLST defined genetic lineages (ST-1, ST17, ST-19 and ST23), with ST-1 and ST-19 being responsible for over 80% of sequence types in colonizing isolates [7]. The sequence polymorphisms at each locus are classified and each allele is given a number. The combination of alleles found in each isolate defines the sequence type, and sequence types having allelic profiles that share at least five or six identical alleles are grouped within a particular clonal complex (CC) [13].

About 60% of all sepsis in preterm neonates is caused by GBS and sepsis is the second most common cause of death in preterm babies (behind lethal malformations) [14]. Prior to 2001, 15 to 50% of neonates systemically infected with GBS die annually. Notably, epidemiological data from the USA consistently mentions a higher incidence of invasive GBS disease in black Americans compared to their white counterparts, although reductions in invasive GBS disease incidence have been observed in both groups [15]. In a report by some workers, only a modest decline in overall incidence was observed in the USA state of Minnesota between 2000 and 2010. However, there have been an increase in incidence since 2010 [16].

A study from Africa that characterized isolates collected from Senegal and Central African Republic showed genotypic overlap between the two regions. In Senegal, CC-1 (32.0%) was significantly higher compared to the Central African Republic (10.2%). In contrast, CC-19 (43.2%) was significantly more common in the Central African Republic compared to Senegal (12.0%; $P < 0.0001$). Despite some differences in the population of colonizing isolates circulating in Senegal and Central African Republic, there was some similarity between the two countries, such as the detection of a rarely described CC-26, in 20.0% and 11.4% of isolates from Senegal and Central African Republic, respectively. It has generally been reported that the prevalence of CC-17, which is mostly associated with invasive disease, remains modest in GBS isolates from colonized pregnant women. In contrast, a study by Davies et al. found no significant difference in the relative frequency of CC-17 when comparing invasive and colonizing isolates ($P = 0.80$) [17]. The higher proportion of CC-17 in colonizing isolates from a Canadian study could be attributed to only serotype III isolates having been characterized. The association between serotype-III and CC-17 has been widely documented by others [18,19].

One million children die each year in low-income countries in the

first 4 weeks of life because of neonatal sepsis, accounting for about 23% of the total number of child birth. In 2015, Nigeria recorded a neonatal death rate of 35 per 1000 live birth (CDC, 2016), sepsis accounting for majority of them. In sub-Saharan African, epidemiological data on maternal group B streptococcus carriage is scarce but necessary to design and implement prevention strategies. Unfortunately, this menace killing our neonates, had been debunked mythically by many communities/tribes and in Yoruba language it is termed 'ABIKU'. Scientifically, neonatal death can be traced to a cause. Illiteracy and poverty level are the major factors responsible for this and data scarcity in this part of world.

The global burden of invasive GBS disease in young infants was summarized in a systematic review, which included 56 studies over the period 2000 and 2011; the majority of which were from European and American countries [20]. This review highlighted striking variability in incidence of invasive GBS disease between and within regions. In a meta-analysis of invasive GBS disease in infants less than 90 days of age, the incidence (per 1,000 live births) was reported as 0.57 (range: 0.00-2.60) in Europe, and 0.67 (range: 0.25-2.13) in the Americas; as low as 0.02 (range: 0.00-0.14) in Asia; and highest in Africa as 1.21 (range: 0.24-1.97).

The low incidences of invasive GBS disease reported across Asia may be an underestimate because a large number (>70%) of deliveries occur outside the health-care settings, possibly resulting in an ascertainment bias with many of the EOGND cases missed at birth [21]. It is likely that the incidence in Africa may also be underestimated. Poor access to microbiology laboratories to confirm invasive GBS disease may also contribute to underestimating the incidence [22]. In a separate review of studies conducted only in low-middle income countries, high incidences were reported in Africa, with South Africa having the highest reported incidence (3.06 cases per 1,000 live births) [23].

A few hospital studies have identified regional GBS colonization rates for pregnant women ranging from 12 to 25.8% [24,25]. In 1993, a small study of 162 pregnant women attending a public antenatal clinic in Toowoomba, Queensland, Canada reported a GBS colonization rate of 16.7%. Based on USA data, McLaughlin and Crowther proposed that 10 to 30% of pregnant women in Australia could be colonized with GBS of the Lower genital tract [26].

Studies in some sub-Saharan African countries like Nairobi, Ghana, Gambia and Cameroon revealed that Group B Streptococcus (GBS) is emerging as the main cause of neonatal sepsis and meningitis. However, in Nigeria, information on GBS disease prevalence remains sparse, hence this study.

Materials and Methods

Ethical consideration

Informed consent for collection of the isolates from the study participants at the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC) with Registration numbers (International IRB/IEC/0004553) and (national NHREC/27/02/2009a) and which were approved by the OAUTHC Ethics and Research Committee on Human Subjects of the Obafemi Awolowo University Teaching

Hospital was obtained. The protocol number for this research (ERC/2016/06/05).

Study population/Area

The study was conducted from June 2016 to January 2017, at a tertiary hospital (Obafemi Awolowo University Teaching Hospitals Complex, OAUTHC) which provides health care to the majority of indigenous Africans living in Ile-Ife, Osun State and its environs.

Inclusion and Exclusion Criteria

Inclusion criteria for maternal participants

- Pregnant women delivering at OAUTHC or referred to this facility from other private facilities or traditional birth facilities.
- Able to understand and comply with planned study procedures.
- Signed written informed consent.

Inclusion criteria for infants

- Infants \leq 24 hours of age

Exclusion Criteria

- Refusal for study participation

Methods for Recruitment of Case subjects

The mother was approached for consent and enrolment of her child and herself on arrival for delivery in the labour ward or theatre in case of caesarean section. The Spouses were approached for the consent in cases where the women were inattentive because of labour pain, for the consent. Appointments were booked with some mothers during ante-natal meetings, expected date of delivery (EDD) was taken and phone calls by the nurse/mid wife on duty were put through when such mothers were in labour.

Sample Collection

Rectovaginal swab from 170 consenting pregnant women and umbilical cord swab samples from corresponding neonates(s) were collected using a sterile cotton-tipped applicator. The method of swabbing was otherwise consistent during the study period. Both vaginal and rectal specimens were collected using micropoint diagnostics tipped swabs. The rectal swab was inserted approximately 2 cm pass the anal verge and rotated against the rectal mucosa. The vaginal swab was inserted approximately 2 cm pass the introitus towards the lower vagina mucosal wall and rotated. Additionally, a swab of the baby's umbilical cord was collected also by inserting the swab 2 cm into the cord immediately after it was cut. All three swabs were inserted into the Stuart transport medium without charcoal and transported to the department of Microbiology laboratory for processing. Swabs collected during normal working hours, on weekends or public holidays were processed within 24 h. Where this was not possible, samples were stored at 2-8°C and processed during laboratory hours the next day.

Isolation of the Organisms

Maternal lower vaginal, rectal swabs and neonatal umbilical swabs were inoculated into Lim broth: Todd-Hewitt broth (2

ml) supplemented with 10 mg/l colistin and 15 mg/l nalidixic acid. The broth was incubated overnight at 37°C for 18-24 h for growth. When growth (turbidity) was noticed, samples were then streaked onto freshly prepared sterile enriched plate media (5% blood agar) and incubated for 24-48 h at 37°C in 5% CO₂ in anaeropack jar (2.5 liter, order no. 50-25, product of Mitsubishi Gas Chemical Company Co. Inc., Japan).

Suspected isolates based on morphology on blood agar were again streaked on GBS brilliance agar (oxid, UK) as it is more sensitive in detecting GBS from rectal swabs and vaginal swabs. Furthermore, GBS brilliance agar (oxid, UK) has a higher specificity and detects non-hemolytic GBS as well [27].

The already prepared GBS brilliance agar (oxid, UK) plates were incubated at 37°C for 18-24 h in aerobic conditions and examined for growth of mauve GBS-like colony morphologies. GBS like colonies were identified, they were subjected to further confirmatory tests, such as the catalase test, the Christie Atkinson Munch-Petersen (CAMP) test and Confirmed GBS isolates were stored at 4°C.

Identification of Isolates

Preliminary identification of bacterial isolates was performed using colonial and morphological characteristics. Isolates were further characterized by physiological characteristics through biochemical reactions. Bacterial isolates were identified using the Bergey's Manual of Determinative Bacteriology. Colonial appearance of isolates on culture media and their relative size, colour, texture, elevation, edge and shape were observed. Gram stain, microscopy analysis, catalase and CAMP tests were conducted to confirm *Streptococcus agalactiae*.

DNA Extraction

A colony of GBS bacteria harbouring the DNA of interest was picked and an overnight broth of the colony was prepared at 37°C for 24 h. The tube containing an overnight broth culture of GBS isolates were vortexed at high speed to re-suspend the cells. One milliliter of the vortexed broth culture of GBS isolate was then transferred into an already labeled eppendorf tube and centrifuged at 13,000 rpm for 10 min. The supernatant in the tube was then discarded and blotted on paper towel. After this, 50 μ l of sterile distilled water was added and vortexed to homogenize the pellets. The tube was then boiled at 100°C for 10 min. After boiling, the tube was again vortexed and centrifuged at 13,000 rpm for 10 mins.

Polymerase Chain Reaction (PCR) Amplification and Detection of PCR Products

Isolates were screened for the detection of resistance genes (*tetM*, *tetO* and *linB*) using Polymerase Chain Reaction (PCR). The selection of the isolates was based on the antibiotic profiles. Primers designed as indicated below were synthesized at Inqaba-Biotechnical industries, Pretoria, South Africa.

Molecular Detection of Resistance genes in *Streptococcus agalactiae* Isolates

All multi drug resistant GBS isolates were tested to detect two

genes for tetracycline resistance, *tetM*, *tetO* and one gene for clindamycin resistance *linB* using a set of specific primers as previously described [28,29]. Primer were tested for specificity by BLAST search on NCBI website.

A 50 µl PCR contained 2.5 mM Tris-HCl pH 8.6, 2.5 mM KCl, 2.5 mM MgCl₂, 5 mM dNTP, 0.5 U *Taq* DNA polymerase Thermo Scientific-Phusion Flash High-Fidelity PCR

Master Mix, AB, Inqaba-Biotechnical Industry, Pretoria, South Africa), PCR grade water, and 1 µM primers pairs forwards and reverses was used. A total of 5 µl template DNA was used in the PCR. The cycling conditions on a My Cycler™ thermal cycler (BioRad Laboratories, London, UK).

Statistical Analysis

Significant differences and relationship between various data obtained were compared using SPSS 20 version.

Results

Ninety eight (98) GBS isolates were recovered from 170 pregnant women and their neonates. 50 (29.4%) of the pregnant women were GBS positive during labour, 31 (18.2%) were GBS positive at rectum alone, 32 (18.8%) were GBS positive in their vagina , 13 (7.6%) were GBS positive in both rectum and vagina while 35 (20.6%) were neonatal umbilical cord GBS positive. 26 (15.3%) were neonatal GBS positive with vertical transmission from their mother, 9 (5.3%) of the neonates were GBS positive without any trace to their mother, 9 subjects had both rectal and vaginal samples positive for GBS as well as their neonate, 9 subjects were positive for GBS in their vaginal as well as their neonates, nine also tested positive to GBS in their rectal sample as well as their neonates.

On demographic parameters (**Table 1**), Pregnant woman Age range 31-35 (31.8%) shows huge validity frequency for GBS followed by age range 26-30 (31.2%) and 41-45 (2.9%) which showed the lowest frequency among the subjects.

Considering religion of the Pregnant women, Christianity representing 84.7% while 15.3% of the subject shows Islam. Marital status of the pregnant women indicates, 99.4% married and 0.6% single.

Tribes of the pregnant women indicated, 88.8% Yoruba, 8.8% Igbo, 1.2% Hausa and 1.2% others which include the other minority tribes.

Occupation of the pregnant women and their husbands indicated, Skilled 48.2 % and 62.4% respectively, Unskilled 23.5% and 20.0% respectively, Semi-Skilled 21.2% and 15.3% respectively while others show 7.1% and 2.4% respectively.

The GBS status of the subjects based on clinical parameters (**Table 2**) shows that 22 out of 41 positive women had fever during pregnancy while the remaining 19 did not. 10 from 27 GBS positive neonates came from mothers who had fever during pregnancy.

Based on previous infection, 18 from 36 representing exactly 50%

Table 1 Frequency table of the Socio- demographic parameters.

Variable	Frequency n=170	Percentage %
Age of Mothers		
15-20yrs	11	6.5
21-25yrs	31	18.2
26-30yrs	53	31.2
31-35yrs	54	31.8
36-40yrs	16	9.4
41-45yrs	5	2.9
Total	170	100
Religion		
Christianity	144	84.7
Islam	26	15.3
Total	170	100
Marital Status		
Single	1	0.6
Married	169	99.4
Total	170	100
Tribe (ethnicity)		
Yoruba	151	88.8
Hausa	2	1.2
Igbo	15	8.8
Others	2	1.2
Total	170	100
Mother's Occupation		
Skilled	82	48.2
Unskilled	40	23.5
Semi-Skilled	36	21.2
Others	12	7.1
Total	170	100
Husband's Occupation		
Skilled	106	62.4
Unskilled	34	20
Semi-Skilled	26	15.3
Others	4	2.4
Total	170	100
Residence LGA		
Ado Ekiti	4	2.4
Ede	1	0.6
Ede South	1	0.6
Efon Alaye	1	0.6
Eleyele	2	1.2
Gbongan	1	0.6
Ibadan	1	0.6
Ido onigbaya	1	0.6
Ife Central	66	38.9
Ife East	60	35.3
Ife North	4	2.4
Ife South	1	0.6
Ila Orangun	3	1.8
Ilesha	16	9.4
Ilesha west	1	0.6
Irewole	1	0.6
Iwo	1	0.6
Ondo west	2	1.2

Oran Ife	1	0.6
Owo	1	0.6
Oye	1	0.6
Total	170	100
Highest level of maternal education		
Primary	5	2.9
Secondary	72	42.4
Tertiary	93	54.7
Total	170	100
Highest level of Paternal education		
Primary	4	2.4
Secondary	64	37.6
Tertiary	102	60
Total	170	100
Parity		
Primigravida (first pregnancy)	61	35.9
Multigravida (multiple pregnancy)	109	64.1
Total	170	100

of GBS colonized mothers had previous infection and 12 from 20 positive neonates were given birth to by mothers with UTI while the remaining 18 positive mothers did not have previous infection and 8 from 20 GBS positive neonates had GBS negative mothers. A huge number of the positive mothers had no history of PROM representing 40 from 46 and 27 from 31 GBS positives neonates came from mothers who did not have premature rupture of membrane. GBS Thirty six from 48 GBS positive mothers had antibiotics administered during labour, 23 from 34 positive neonates came from mothers who had antibiotics during labour. Birth weight classification shows that 24 from 50 GBS positive mothers had neonates with birth weight of 2.0-2.99 kg. Mode of delivery shows 15 from 49 GBS positive mothers had caesarean section and 34 from 49 had Spontaneous vaginal delivery (SVD). 12 from 34 positive neonates were born through caesarean section and 22 through SVD.

31 vaginal and 32 rectal samples were positive to GBS, 50 rectovaginal sample altogether were positive to GBS indicating that the presence of GBS in either rectal or vaginal is considered rectovaginal positive and this yielded the 29.4% GBS prevalence. The GBS status of the subjects based on clinical parameters (**Table 3**). Twenty two (22) from 41 positive women had fever during pregnancy while the remaining 19 did not. 10 from 27 positive neonates came from mothers who had fever during pregnancy while the remaining 17 didn't.

35 from 170 neonatal umbilical cord specimens were positive representing 20.6% as shown in **Table 3**.

The test of association (using the Pearson chi-square) between demographic and clinical parameters versus the GBS status of the subjects is shown in **Table 4**. The test of association between marital status and GBS status indicated that 32 positive vaginal samples accounting for 18.8% were from married women, all the 31 positive rectal samples accounting for 18.2% were also

married, all the 35 positive neonatal samples were neonates from married women as well as the 50 maternal samples (29.6%) were from married women. P- values from the tables >0.05.

Table 2 Frequency table of the clinical parameters.

Variable	Frequency n=170	Percentage %
Previous Infection		
Yes	64	37.6
No	59	34.7
Total	123	72.4
Missing values	47	27.6
Total	170	100
History of fever		
Yes	58	34.1
No	86	50.6
Total	144	84.7
Missing values	26	15.3
Total	170	100
History of PROM		
Yes	28	16.5
No	129	75.9
Total	157	92.4
Missing values	13	7.6
Total	170	100
Administration of Antibiotics during labour		
Yes	39	22.9
No	120	70.6
Total	159	93.5
Missing values	11	6.5
Total	170	100
Caesarean Section		
Yes	47	27.6
No	116	68.2
Total	163	95.9
Missing values	7	4.1
Total	170	100
Sex of Neonate		
Male	96	56.5
Female	74	43.5
Total	170	100
Birth Weight		
1	12	7.1
2	53	31.2
3	87	51.2
4	17	10
Total	166	97.9
Missing Values	4	2.4
Total	170	100
Full length of the Neonate		
1	3	1.8
2	51	30
3	12	7.1
Total	66	38.8
Missing values	104	61.2
Total	170	100
Head circumference of the Neonate		

26	1	0.6
28	1	0.6
30	1	0.6
32	4	2.4
33	10	5.9
34	22	12.9
35	18	10.6
36	3	1.8
37	4	2.4
38	2	1.2
Total	66	38.8
Missing values	104	61.2
Total	170	100
Vaginal GBS colonization		
Positive	32	18.8
Negative	138	81.2
Total	170	100
Rectal GBS colonization		
Positive	31	18.2
Negative	139	81.8
Total	170	100
Neonatal GBS colonization		
Positive	35	20.6
Negative	135	79.4
Total	170	100
Rectovaginal GBS colonization		
Positive	50	29.4
Negative	120	70.6
Total	170	100

The test of association between tribe of the subjects and GBS status. 27 positive vaginal samples accounting for 17.9% of Yoruba women, 3 positive vaginal samples accounting for 20.0% of Igbo. 28 (out of 31) positive rectal samples accounting for 18.5% of Yoruba, 32 (out of 35) positive neonatal samples accounting for 21.2% of neonates born to Yoruba women as well as the 44 (out of 50) maternal samples (29.1%) were neonates born to Yoruba women. Only rectal had a P- values <0.05 from the tables.

However, the test of association shows statistical significance with p value <0.05 in the following parameters as shown in the **Table 4**. The test of tribe versus vaginal and rectal as separate variables had value of 0.027 and 0.013 respectively. Wife's occupation versus rectal had a value of 0.05, husband's occupation vs rectal and neonate had values of 0.011 and 0.05 respectively.

Wife's level of education and rectal colonization with a p-value of 0.045, husband's level of education versus vaginal colonization with p-value of 0.043. Previous infection versus rectal colonization with p-value of 0.020. History of fever during pregnancy with p-value of 0.004. Birth weight versus neonatal and rectovaginal colonization with 0.004.

Molecular Detection of *tetM*, *tetO* and *linB* Resistance Genes in GBS Isolates

The agarose gel electrophoresis of *tetM* is shown in **Figure 1**. One of the 8 isolates that were resistant to antibiotics harboured

Table 3 GBS colonization based on demographic parameters and clinical parameters.

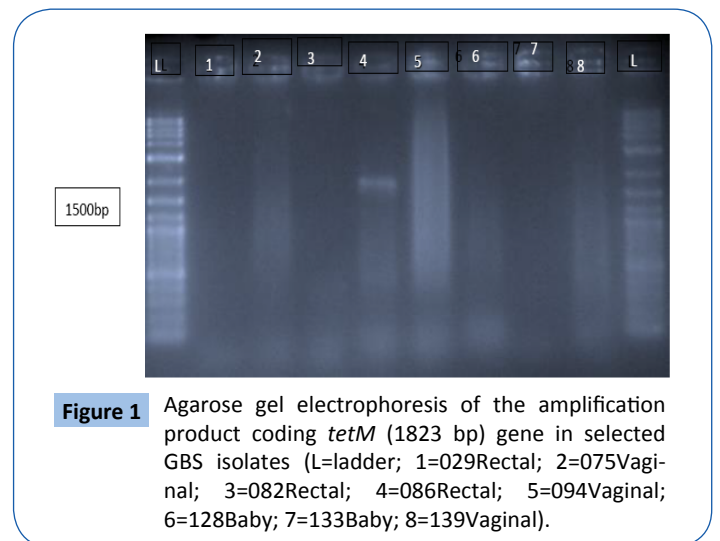
Demographic parameters and clinical parameters				
Religion				
Christianity	144	39 (29)	105 (115)	
Islam	26	11 (6)	15 (20)	
Traditional	0	0	0	
others	0	0	0	
Total	170	50	120	0.117 (0.733)
Marital Status				
Married	170	50	120	
Single	0	0	0	
Cohabiting	0	0	0	
Divorced	0	0	0	
Total	170	50	120	0.517 (0.610)
Tribe				
Yoruba	151	44 (32)	107 (119)	
Hausa	2	0 (0)	2 (2)	
Ibo	15	4 (2)	11 (13)	
others	2	2 (1)	0 (1)	
Total	170	50	120	0.128 (0.553)
Mother's occupation				
Unskilled	40	9 (7)	31 (33)	
Semi-Skilled	36	12 (5)	24 (31)	
Skilled	82	25 (20)	57 (62)	*
Others(Students)	12	4 (3)	8 (9)	
Total	170	50	120	0.724(0.554)
Father's occupation				
Skilled	106	38 (28)	68 (78)	*
Unskilled	34	4 (5)	30 (29)	
Semi-Skilled	26	7 (1)	19 (25)	
Others(Students)	4	1 (1)	3 (3)	
Total	170	50	120	0.062 (0.590)
History of Fever				
yes	58	22 (10)	36 (48)	
No	86	19 (17)	67 (69)	
Total	107	29	78	0.039(0.703)
Previous Infection				
Yes	64	18 (12)	46 (52)	
No	59	18 (8)	41 (51)	
Cross tab neonates birth weight Mother (neonate)				
Birth weight				
0.0-1.99	12	1 (3)	11 (9)	
2.0-2.99	53	24 (19)	29 (34)	
3.0-3.99	87	21 (9)	66 (78)	
4.0 and Above	17	4 (4)	13 (13)	
Total	169	50	119	0.015 (0.004)
History of PROM				
Yes	28	6 (4)	22 (24)	
No	129	40 (27)	89 (102)	
Total	157	46 (31)	111 (126)	0.313 (0.423)
Antibiotics during Labour				
Yes	39	12 (11)	27 (28)	
Parity				
Primi gravida	61	21 (10)	40 (51)	
Multi gravida	109	29 (25)	80 (84)	

Total	170	50 (35)	120 (135)	0.283 (0.312)
Sex of Neonate				
Male	96	23 (17)	73 (79)	
Female	74	27 (18)	47 (56)	
Total	170	50 (35)	120 (135)	0.076 (0.290)
Antibiotics during Labour				
Yes	39	12 (11)	27 (28)	
No	120	36 (23)	84 (97)	
Total	159	48 (34)	111 (125)	0.928 (0.232)
Caesarean section				
Yes	47	15 (12)	32 (35)	
No(SVD)	116	34 (22)	82 (94)	
Total	163	49 (34)	114 (129)	0.743 (0.350)
Neonate's Full Length				
30-39	3	0 (1)	3 (2)	
40-49	51	15 (12)	36 (39)	
50-59	12	7 (6)	5 (6)	
Total	66	22 (19)	44 (47)	0.073 (0.183)
Parity				
Primi gravida	61	21 (10)	40 (51)	
Multi gravida	109	29 (25)	80 (84)	
Total	170	50 (35)	120 (135)	0.283 (0.312)
Sex of Neonate				
Male	96	23 (17)	73 (79)	
Female	74	27 (18)	47 (56)	
Total	170	50 (35)	120 (135)	0.076 (0.290)

Table 4 Test of association between variables (demographic parameters and clinical history) and GBS status of the subjects.

Variable	Total, n	GBS Colonized	GBS non-Colonized	p- value
Tribe vs Vaginal GBS colonization				
Yoruba	151 (100%)	27 (17.9%)	124 (82.1%)	
Hausa	2	0	2	
Igbo	15 (100%)	3 (20%)	12 (80%)	
Others	2 (100%)	2 (100%)	0 (0%)	
Total	170	32	138	0.02<0.05
Tribe vs Rectal GBS colonization				
Yoruba	151 (100%)	28 (18.5%)	123 (81.5%)	
Hausa	2	0	2	
Igbo	15	1 (6.7%)	14 (93.3%)	
Others	2	2 (100%)	0	
Total	170	31	139	0.013<0.05
Wife's occupation vs Rectal GBS colon				
Skilled	82 (100%)	21 (25.6%)	61 (74.4%)	
Unskilled	40	3 (17.5%)	37	
Semi-Skilled	36	4 (11.1%)	32	
Others (students)	12	3 (25%)	9	
Total	170	31	139	0.05 ≤ 0.05
Husband's occupation vs Rectal GBS colon				
Skilled	106 (100%)	27 (25.5%)	79 (74.5%)	
Unskilled	34	1 (2.9%)	33 (97.1%)	
Semi-Skilled	26	2 (2.9%)	24 (97.1%)	
Others (students)	4	1 (25%)	3 (75%)	
Total	170	31	139	0.011<0.05

Variable	Total, n	GBS Colonized	GBS non-Colonized	p- value
Tribe vs Vaginal GBS colonization				
Husband's occupation vs neonate GBS colon				
Skilled	106 (100%)	28 (26.4%)	78 (73.6%)	
Unskilled	34	5 (14.7%)	29 (85.3%)	
Semi-Skilled	26	1 (3.8%)	25 (96.2%)	
Others (students)	4	1 (25%)	3 (75%)	
Total	170	35	135	0.05 ≤ 0.05
Wife's L. Education vs Rectal GBS colon				
Primary	5 (100%)	0 (0)	5 (100%)	
Secondary	72	8 (11.1%)	64 (88.9%)	
Tertiary	93	23 (24.7%)	70 (75.3%)	
Total	170	31	139	0.045<0.05
Husband L. Education vs Vaginal GBS colon				
Primary	4 (100%)	0 (0)	4 (100%)	
Secondary	64	18 (28.1%)	46 (71.9%)	
Tertiary	102	14 (13.7%)	88 (86.3%)	
Total	170	32	138	0.043<0.05
Previous infection (UTI) vs Vaginal GBS colonization				
Yes	64 (100%)	17 (26.6%)	47 (73.4%)	
No	59	6 (10.2%)	53 (89.8%)	
Total	123	23	100	0.020<0.05
History of Fever vs Vaginal GBS colon				
Yes	58 (100%)	17 (29.3%)	41 (70.7%)	
No	86 (100%)	9 (10.5%)	77 (89.5%)	
Total	144	26	118	0.004<0.05
Birth weight vs neonate GBS colonization				
0.0-1.99	12	3 (25%)	9 (75%)	
2.0-2.99	53	19 (35.8%)	34 (64.2%)	
3.0-3.99	87	9 (10.3%)	78 (89.7%)	
4.0 and Above	17	4 (23.5%)	13 (76.5%)	
Total	169	35	134	0.004<0.05



tetM resistant gene of molecular weight of 1823 bp while none of the 8 isolates harboured *tetO* gene at 1764 bp.

Discussion

Data in this findings provided knowledge regarding GBS and the potential burden. Our study provided basis for further investigation into prevalence of GBS among pregnant women and their neonates. It was identified that up to one in three pregnant women in labour at OAUTHC and one in five neonates within 24 h of birth tested positive to GBS colonization. The overall prevalence among 170 pregnant women age 18-45 years was 29.4% and 20.6% in neonates a higher rate than previously reported maternal colonization rate of 11.3 % by Onipede et al. 18% prevalence in Enugu, eastern Nigeria [30], 19% among Ethiopian women reported by Mengist et al. and Le Doare et al. reported a prevalence of 33.73% in pregnant women and 24.8% in Neonates among Gambians [31].

Pregnant women between age 26 and 35 years are at greater risk of GBS colonization, considering that age group as peak childbearing age. In this study, the age group with the highest colonization rate was 15-20 with 36.4% while 26-30 and 31-35 had 32.1% and 27.8% respectively, showing that even younger women are at greater risk of GBS colonization. Although, age group 15-20 had the highest colonization rate, the risk of vertical transmission to neonate is low with 9.1%. However, age groups 26-30 and 31-35 had the highest transmission rate to their neonate with 26.4% and 27.8% respectively.

Statistically, there is no significant association between age, religion, marital status, sex of neonate, parity and GBS colonization of mother and neonate. Onipede et al. also reported no significant association between GBS colonization and age, gestational age, gravidity and obstetric risk (p value >0.05) [32]. However, 42.3% of Muslim mothers were GBS colonized while 27.1% of Christian mothers showing a higher prevalence among Muslim mothers and a slight difference among GBS colonized neonates with 23.1% and 20.1% respectively.

The route of GBS infection and occupation of the parents had a significant association with p value of <0.05, 25.6% of the skilled mothers and 25.5% of skilled fathers (Doctors, lawyers, civil servants etc.) tested positive to GBS with samples collected from the rectal. 25% in the category "others" (students, traders and businessmen/women) also tested positive to GBS with rectal samples. This two groups also had the highest transmission rate to neonate with 20% and 25% respectively. The Overall GBS colonization seem to be more significant with the semi-skilled (artisans) and the "others" group mothers with 33.3% each and their husbands in skilled (civil servants etc.) and "others" groups. In this study, previous UTI and fever during pregnancy had significant association with vaginal GBS colonization (p values <0.05) with 26.6% and 29.3% positive to GBS respectively, although the causal organism in previous UT infection was not recorded. Patients who delivered through caesarean section had more rectal GBS colonization but less vaginal colonization than SVD. 25.5% of GBS colonized neonates were delivered through CS compared with 19% of neonates from SVD.

Mullaney reported GBS as a nosocomial infection which correlates with this study. Nine (9) from the thirty-five (35) GBS

colonized neonates had no link to the maternal GBS status, which suggests the neonates GBS status could have been acquired from the hospital environment [33].

Interesting, eight (8) from the nine (9) were delivered through SVD which suggest the labour ward as the link between this GBS infected neonates, which can be the origin of late-onset GBS disease.

Hanley, reported low birth weight in neonates with GBS colonized mothers, however, in this study birth weight and anthropometric parameter at birth had significant association with GBS colonization. As high as 45.3% of GBS colonized mothers and 35.8% of GBS colonized neonates had neonatal birth weight of 2.00-2.99, recording p values <0.05 in both cases. 58.3% of GBS colonized mothers and 50% GBS colonized neonate had their full length 50-59, taking a very significant percentage of the GBS colonized mother and neonate. More than 50% of both GBS colonized mother and neonate had Head circumference between 33 and 36.

This study established that GBS had a prevalence of 29.4% among pregnant women and 20.6% among neonates in Nigeria, which is in conjunction with research works from other Africa countries. Pregnant women have been found to be asymptomatic to GBS colonization, however, it has been implicated as a causal organism for sepsis in neonates which is the leading cause of neonatal death worldwide.

The resistant genes amplified (*tetO and LinB*) were absent in the isolates, suggesting the absence of these genes in the environs.

Recommendation

Data from this study provides important epidemiological information on GBS colonization among pregnant women and their neonates in Nigeria and one of the first studies to report GBS prevalence in neonates and vertical transmission (maternal) in Ile-Ife and Nigeria at large. The study reveals high carriage rate of GBS among pregnant women compare to some previous studies in the country. More than one-fourth of pregnant women, one-fifth of neonates born within 24 h harbored GBS. Our study highlighted that all age groups among pregnant women in Ile-Ife is potentially at risk of GBS colonization and pregnant women <20 years are at the greatest risk of GBS colonization with up to one in three, neonates are at risk of nosocomial GBS colonization and those born with SVD are at greater risk. Neonates born with a negative GBS status can acquire it in the hospital. Nosocomial being responsible for about 5% of GBS colonization among neonates.

Further research is called for using larger sample size and multiple curve studies for adequate extrapolation into the general population. This is also an eye opener for health policy makers in Nigeria to look into maternal and neonatal GBS colonization for possible review in National Health Policy. Reductions in GBS infant and neonatal disease will occur when a screening based consensus protocol is implemented. Screening provides clinicians with a maternal GBS colonization results which provides a basis for early maternal preventative treatment options. When non-screening based prevention protocols are implemented, the

clinician must rely on previously reported risk factors associated with maternal GBS carriage or obstetric risk factors being symptomatic during labour.

Authors Contribution

All contributing authors have agreed to the submission of this

manuscript for publication. Omololu-Aso J conceived, formulate the hypothesis in designing of project, supervised and wrote the paper. Akinlolu JT performed the experiments, analyzed the data and interpreted the results. Owolabi AT Co-supervised and analyzed the findings. Omololu-Aso OO formulates the hypothesis in designing of project and edited the manuscripts.

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