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Streptococcus agalactiae colonization is common among pregnant women with HIV infection and is neither predicted by hospital tier nor trimester in Mwanza, Tanzania

Paul H. Ngayomela¹, Richard Kiritta¹, Boniface A. Remi², Sophyrose T. Mboya², Nyambura Moremi³, Dismas Matovelo¹, Fridolin Mujuni¹, Stephen E. Mshana², Claudia Daubenberger^{4,5}, Ruth N. Zadoks^{6,7} and Jeremiah Seni^{2*}

Abstract

Background *Streptococcus agalactiae*, also known as Group B *Streptococcus* (GBS) is a member of the gastrointestinal tract and vaginal microbiota, and one of the commonest pathogens affecting pregnant women, neonates and infants. Its observed rate of colonization varies globally due to laboratory methods used, socio-cultural, epidemiological and clinical factors. This study therefore, aimed at determining the prevalence of GBS colonization and associated risk factors among HIV-infected and uninfected pregnant women in various health care facility tiers in Tanzania to guide priority screening and management.

Methods A cross-sectional analytical study was conducted from February to June 2021 from the antenatal clinics in the primary, secondary and tertiary health care levels in the northwestern part of Tanzania involving 872 pregnant women. Demographic, obstetric and other clinical data were collected using a pre-tested structured questionnaire. Two swabs (vaginal and rectal) were collected and cultured on blood agar and CHROMagarTMStrepB agar, followed by antimicrobial susceptibility testing. A two-sample test of proportions was used to compare the GBS prevalence in various sub-groups, and logistic regression analysis was deployed to ascertain the association between predictor variables and GBS colonization.

Results The overall proportion of *S. agalactiae* colonization was 24.5% (214/872) [95% CI: 21.7 – 27.5%], and was significantly higher in the HIV infected group [63.1% (70/111)] than in the HIV-negative group [18.9% (144/761)]; OR (95% CI) = 7.33 (4.77–11.29, p-value < 0.001). Colonization was more prevalent in the rectal samples compared to vaginal samples [21.1% versus 8.83%, respectively; p-value < 0.001] and *S. agalactiae* recovery was higher using CHROMagarTMStrepB agar compared to blood agar [24.4% versus 18.8%, respectively, p-value = 0.004]. There was no association between GBS colonization and health care facility levels or pregnancy trimesters. The *S. agalactiae* isolates were sensitive to ampicillin (100%), erythromycin (76.3%), clindamycin (84.4%) and vancomycin (98.1%).

*Correspondence:
Jeremiah Seni
senijj80@gmail.com

Full list of author information is available at the end of the article



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Conclusions Approximately a quarter of pregnant women are colonized by GBS in Mwanza, Tanzania; and colonization is remarkably higher among HIV-infected women. Ampicillin is recommended for prophylaxis and treatment of GBS. Introduction of routine GBS screening among all HIV-infected pregnant women using ultrasensitive CHROMagar™ Strep B agar is recommended, and molecular characterization of GBS isolates would be of interest to guide future vaccination strategies.

Keywords *Streptococcus agalactiae* colonization, HIV status among pregnant women, Drug susceptibility patterns

Background

Streptococcus agalactiae, also known as Group B *Streptococcus* (GBS) is one of the leading causes of infections in pregnant women, infants and neonates [1, 2]. Globally, the incidence of systemic invasive GBS infection in pregnant women is estimated at 0.38 cases per 1,000 pregnancies, with an approximately case fatality rate of 0.2% [1, 2]. The bacteria may exist as part of the normal microbiota in the genital tract and anal-rectal parts of healthy adults, with the latter serving as a natural reservoir and source of vaginal colonization in women [3, 4]. Maternal *S. agalactiae* colonization in various sites predisposes newborns to colonization and adverse neonatal outcomes like preterm births, low birth weight, neonatal sepsis, neonatal encephalopathy and deaths [5–9]. Although *S. agalactiae* has the ability to cross through the intact amniotic membrane and subsequently result in intrapartum fetal infections, the predominant route of infection is thought to be through GBS transfer to the neonates as it passes through the birth canal, which may result in serious infections with high mortality rate [10].

Estimates from a systematic review and meta-analysis showed an overall prevalence of *S. agalactiae* colonization during pregnancy of 17.9%, with the prevalence being relatively lower in the Americas (19.7%), Europe (19.0%) and Southern Asia (11.1%) compared sub-Saharan Africa 22.4% [10, 11]. A recent systematic review and meta-analysis of maternal GBS colonization in sub-Saharan Africa highlighted the imbalanced nature of available data from the region, with more than half of the available studies originating from just three countries (Ethiopia, Nigeria and South Africa). Furthermore, the immediate need for screening of pregnant women at health care facilities has raised pertinent questions about suitability of screening methods in the context of African health care facilities; and concerns about antimicrobial resistance, whereby approximately a quarter of GBS isolates were resistant to erythromycin, a safe antibiotic during pregnancy which is most often used as an alternative to beta lactam antibiotics like penicillin [12]. Another important consideration in many African countries is that a few studies have shown GBS colonization to be higher among HIV-infected pregnant women than those who are not infected, for instance, 24% versus 13.7% in Ethiopia [13], and 50% versus 19.1% in the Democratic Republic of Congo [14]. The two Tanzanian studies that

were included in the meta-analysis were conducted in Muhimbili National Hospital (MNH) and Bugando Medical Centre (BMC), and showed the prevalence of GBS to be 23% and 9.5%, respectively, suggesting the possibility of considerable differences even within countries. Of note, in the MNH study, swabs were enriched in Todd-Hewitt broth containing nalidixic acid and gentamicin, followed by inoculation onto conventional blood agar media, whereas in the BMC study, swabs were directly inoculated onto the conventional blood agar media [9, 15]. Low sample sizes in these studies among HIV-infected women, dependent on conventional culture with low bacterial yield (as opposed to ultrasensitive media like CHROMagar™ StrepB), confinement to tertiary hospitals and the fact that they were conducted over a decade ago have altogether limited plausible extrapolation of the findings in the current context [9, 15, 16]. Building on a similar study on urinary tract infections which spanned from primary health care facilities (health centers and district hospitals), through regional referral hospital to tertiary hospital in the north-western part of Tanzania [17], the current study was designed to address several research and implementation gaps, by considering GBS detection using multiple methods across three tiers of health care facilities, followed by antimicrobial susceptibility testing, to inform specific preventive measures and expedite provision of GBS antimicrobial prophylaxis. Moreover, stratification of GBS colonization among pregnant women by HIV infection status was considered to ascertain whether both groups, or only those with HIV infection should be prioritized in the future GBS screening and vaccination programs.

Materials and methods

Study design and settings

This was a cross-sectional analytical study comparing maternal vaginorectal *S. agalactiae* colonization between HIV-infected and uninfected pregnant women from February to June 2021. The study was conducted across multiple tiers of the health care referral system in the northwestern part of Tanzania from primary and secondary to tertiary levels. The primary health care facilities involved were Makongoro Health Centre (MHC) in the urban area, Karume Health Centre (KHC) in the rural area, Sengerema District Designated Hospital (SDDH) in the rural area and Nyamagana District Hospital (NDH)

in the urban area. Selection of these facilities was based on the large number of pregnant women attending antenatal clinics (ANC), and rural-urban representation. The secondary level health care facility was Sekou Toure Regional Referral Hospital (SRRH), and the tertiary level health care facility was Bugando Medical Centre (BMC). The latter two are the only secondary and tertiary facilities in Mwanza region.

Inclusion and exclusion criteria

All pregnant women (HIV infected, and non-HIV infected) at any gestation age residing in Mwanza region who consented to participate were included in the study. Pregnant women with obstetric complications such as vaginal bleeding or cervical cerclage in situ (as shown in the attending doctor's captioned pregnant women's hospital medical records), and pregnant women who were coming from outside Mwanza region were excluded. The limitation to only Mwanza region was meant to reduce participants' heterogeneity, and foster regional-level utilization of study findings. In Tanzania, HIV testing among pregnant women is done routinely in the ANC using serial SD Bioline and Uni-Gold HIV serological tests, and in an event the two serological tests are discordant, either ELISA or HIV RNA/DNA Real Time Polymerase Chain Reaction is done for confirmation purposes. The results are recorded in the ANC card for every pregnant woman. In addition, for those found to be HIV infected, CD4 + T Lymphocyte count and HIV viral load are also measured. All HIV-infected pregnant women are given antiretroviral therapies irrespective of their immunological status [18].

Sample size and sampling procedures

We calculated the sample size to detect a significant difference between two proportions, i.e., the prevalence of GBS in HIV-positive women and HIV-negative women, using EpiTools (<https://epitools.ausvet.com.au/twoproporitions>). Inputs into the sample size calculation included the estimated GBS prevalence in HIV-negative women, calculated as the mean of observed GBS prevalence for this population in Tanzania (Muhimbili, 8.3%) and Ethiopia (13.7%) [9, 13]; the estimated GBS prevalence in HIV-positive women, was again calculated as the mean of observed GBS prevalence in Tanzania (24.3%) and Ethiopia (24%); the desired confidence level (0.9) and power (0.8); and the ratio of HIV-negative to HIV-positive women based on data from Tanzania (276:24 or 11.5 [9, 13]), giving a minimum total sample size across both groups of 850, including 68 HIV-positive women and 782 HIV-negative women. The number of pregnant women enrolled per health care facility was computed proportionately taking into account variability in the hospital tiers, obstetric bed capacities, and the number

of HIV infected women attending ANC. The recruitment of clients was serially done in two arms namely HIV infected pregnant women from the Prevention of Mother to Child Transmission of HIV (PMTCT) Clinics, and HIV uninfected pregnant women in the routine ANC. A total of 32 patients were excluded [obstetrics complications ($n=8$), did not consent ($n=18$) and non-Mwanza residents ($n=6$)]. Data were collected using a pre-structured questionnaire, and whenever there was any missing data, it was tracked from the electronic Hospital Management Information System (eHMIS). The final number of enrolled pregnant women was 872. The distribution of pregnant women enrolled from the 'higher level' health care facilities (BMC and SRRH) were 211 and 102, respectively; while enrollment from 'lower level' health care facilities (NDH, SDDH, MHC and KHC) were 147, 141, 136 and 135, respectively. The respective HIV-infected pregnant women enrolled in these health care facilities were 45, 4, 16, 17, 11, and 18.

Data collection

Comprehensive history taking from all participants using a structured pre-tested questionnaire was used for the data collection. The questionnaire was piloted in a health center that was not part of the study sites to ascertain its validity, clarity and comprehensibility. Data collection was done by the first author (PHN), trained residents and research assistants, and was reviewed routinely by senior project team members to ensure reliability using the existing HMIS. The questionnaire contained questions on socio-demographic characteristics, obstetric history and other clinical information. These included maternal age, residence, gestation age, gravidity, parity, HIV status, history of abortions, occupation, marital status, recent use of antibiotics, and history of admission (among others). The variables were selected based on previously published articles on the subject matter, and existing variables in the BMC HMIS.

Specimen collection and laboratory procedures

Sterile microbiology transport swabs (IMPROSWAB®, Guangzhou, China) were used to collect vaginorectal swab specimens from all pregnant women who consented following standard procedures. The attending doctor or obstetrician put on a sterile glove, separated labia, and a swab was inserted 2–3 cm into the vagina and rotating it with a circular motion without a speculum, leaving it in the vagina for approximately five seconds, and then, a vaginal swab was taken. A separate rectal swab was also taken by gently rotating the swab around the anal margin for approximately five seconds without use of any gel. Swabs were placed in Amies Transport media, and transported to the laboratory within 2 h for processing according to standard operating procedures [19]. Samples were

recorded and cultured on blood agar (Oxoid, UK) and CHROMagarTMStrepB (CHROMagar, Paris, France). CHROMagarTMStrepB was chosen based on its high sensitivity and positive predictive value of 100.0% as reported in previous studies [16, 20]. Media were incubated at 35 to 37 °C for 18 to 24 h. Identification of GBS was based on bacterial morphology, including beta-hemolysis on blood agar or distinctive mauve to purple colonies on the CHROMagarTMStrepB, Gram staining microscopy (i.e. gram- positive cocci single, in pairs or chains), and negative results in catalase testing. Antimicrobial susceptibility testing for all GBS isolates was done on Mueller Hinton Agar (Oxoid, UK) using the disk diffusion method as previously described by the Clinical Laboratory Standard Institute (CLSI) [21]. Results for culture and antimicrobial susceptibility testing were made available to the participating women and attending doctors within three to seven days to guide specific management in accordance with the respective hospital guidelines. GBS colonization in a pregnant woman was defined as a positive result from a vaginal or rectal swab or both on blood agar, CHROMagarTMStrepB media, or both.

Data analysis

Data entry was done using Microsoft Excel and exported to STATA version 13 for analysis. Continuous variables were summarized using mean with standard deviation or median with the interquartile range depending

on the distribution. Categorical variables were summarized using frequency and proportion (percent). A two-sample test of proportions was used to compare the GBS prevalence between vaginal and rectal samples, and also between blood agar and CHROMagarTMStrepB. Association between independent variables (socio-demographic, obstetric, and clinical characteristics like HIV infection status) and outcome variables (GBS colonization) were determined by logistic regression analysis and the strength of the association was expressed using the odds ratio (OR) with 95% confidence interval (95% CI) and a p-value of <0.05 to declare significance. In addition, any variables with a p-value of <0.2 in univariable analysis were subjected to multivariable analysis to determine the independent predictors of GBS colonization. Multicollinearity was also carefully assessed prior to subject variables to the multivariable analysis, for example, “health care facility tier”, and “referral status” are related because referred patients are usually sent to the high tier health care facility.

Results

Socio-demographic and clinical characteristics of the study participants

The median age (IQR) of study participants was 26 (22–30) years, with the youngest and oldest pregnant woman being 17 years and 48 years, respectively. The majority of participants were Mwanza city dwellers, 80.2% ($n=699$), with the rest enrolled from other districts within Mwanza region (Table 1). Approximately 60% of participants were in the third trimester. A total of 111 (12.7%) were HIV infected. The majority of HIV infected pregnant women were on ART, 94.6% (105/111), however, only 30 (27.0%) were on trimethoprim-sulphamethoxazole prophylaxis. Of 111 HIV infected pregnant women, only 17 (15.3%) had their CD4+ T lymphocyte count recorded, and the median count (IQR) was 563 (454–661). Similarly, the HIV viral load was recorded in 6 participants only. A total of 93 (10.7% of 872) participants had a history of antimicrobial use in the past month with the majority of these being on penicillin, amoxycillin or ampicillin, 78 (83.9% of 93) (Table 2).

Prevalence of *S. agalactiae* vaginorectal colonization by sample type and culture media

The overall proportion of *S. agalactiae* colonization was 24.5% (214/872) [95% CI: 21.7 – 27.5%], with some differences between individual health care facilities tiers were; BMC 28.0%, 59/211; SRRH 18.6%, 19/102; NDH 29.3%, 44/147; SDDH 19.2%, 27/141; MHC 22.8%, 31/136; KHC 25.2%, 34/135. *S. agalactiae* was detected significantly more commonly in the rectal samples compared to vaginal samples [21.1% (184/872) versus 8.83% (77/872), respectively; p-value <0.001] (Table 3). Of all women

Table 1 Socio-demographic characteristics of the study participants

Patient Characteristics (N=872)	Frequency (n)/Median (IQR)*	Percentage (%)
Age	26 [22–30] *	
Marital status		
Married	667	76.5
Not Married	205	23.5
Residence		
City	699	80.2
Outside city	173	19.8
Education level		
Informal	58	6.7
Primary	428	49.1
Secondary	282	32.3
College	62	7.1
University	42	4.8
Occupation		
Housewife	235	27.0
Peasant	249	28.5
Informal business	242	27.7
Employed	100	11.5
Formal business	46	5.3

*Continuous variable

Table 2 Clinical and obstetrics information of study participants

Patient characteristics (N = 872)	Frequency (n)/ Median (IQR)*	Per- cent- age (%)
Gravidity		
Primegravida	253	29.0
Multigravida	466	53.4
Grandmultigravida	153	17.6
Parity		
Nullipara (0)	313	35.9
Para (1–4)	509	58.4
Grandparity (≥ 5)	50	5.7
History of abortion		
Yes	712	81.7
No	160	18.3
Gestation Age		
1st trimester	28	3.2
2nd trimester	318	36.5
3rd trimester	526	60.3
HIV Status		
Positive	111	12.7
Negative	761	87.3
VDRL in the ANC*		
Positive	3	0.3
Negative	869	99.7
MRDT in the ANC		
Positive	16	1.8
Negative	856	87.2
Haemoglobin level (g/dl) in the ANC*	11.4 [10.5–12.3]	
Temperature(°C)*	36.5 [36.2–36.9]	
Family history of DM		
Yes	26	3.0
No	846	97.0
Maternal history of DM		
Yes	10	1.1
No	862	98.9

ANC: Antenatal clinic card; HIV: Human immunodeficiency virus; VDRL: Venereal Diseases Research Laboratory test for syphilis; MRDT: Malaria Rapid Diagnostic Test; DM: Diabetes mellitus; *Continuous variable

Table 3 Comparison of *Streptococcus agalactiae* isolation by sample type irrespective of culture media

		<i>Streptococcus agalactiae</i> isolation from vaginal samples		
		Positive	Negative	Total
<i>Streptococcus agalactiae</i> isolation from rectal samples	Positive	46	138	184
	Negative	31	657	688
	Total	77	795	872

diagnosed with *S. agalactiae*, only 21.4% (46/215) were positive on both swabs, whereas the majority were positive on a single swab only [64.8% (138/215) versus 14.4% (31/215) for rectal or vaginal swab, respectively]. *S. agalactiae* recovery from bacteriological media was significantly more in CHROMagar™ Strep B agar compared to

Table 4 Antimicrobial susceptibility of *Streptococcus agalactiae* isolated from pregnant women in Mwanza, Tanzania, 2021

Antibiotics	Sensitivity of vaginal isolates (%)	Sensitivity of rectal isolates (%)	Overall sensitivity (%)
Ampicillin	79 (100.0)	178 (100.0)	257(100.0)
Tetracycline	2 (2.5)	4 (2.3)	6 (2.3)
Clindamycin	71 (89.9)	146 (82.0)	217 (84.4)
Erythromycin	63 (79.8)	133 (74.7)	196 (76.3)
Vancomycin	77 (97.5)	175 (98.3)	252 (98.1)
Azithromycin	61 (77.2)	142 (79.8)	203 (79.0)
TMP-SXT	5 (6.3)	2 (1.1)	7 (2.7)
Ciprofloxacin	75 (94.9)	173 (97.2)	248 (96.5)
Total number of isolates	79*	178**	257

TMP-SXT: Trimethoprim-sulfamethoxazole; *Two isolates were in both vaginal and rectal samples; ** Four isolates were in both vaginal and rectal samples.

blood agar [24.4% (213/872) versus 18.8% (164/872), respectively, p-value = 0.004]

Antimicrobial susceptibility patterns of *S. agalactiae* isolated from pregnant women in Mwanza, Tanzania

The *S. agalactiae* isolates were all sensitive to ampicillin (100%) irrespective of the type of sample. They were also sensitive to erythromycin (76.3%), clindamycin (84.4%) and vancomycin (98.1%). Very few isolates were susceptible to tetracycline (2.3%) or trimethoprim-sulphamethoxazole (2.7%) (Table 4).

Risk factors associated with *S. agalactiae* colonization among pregnant women

S. agalactiae colonization was significantly more prevalent in the HIV infected pregnant women compared to HIV-negative pregnant women [63.1% (70/111) versus 18.9% (144/761)], respectively; OR (95% CI) = 7.3 (4.8–11.2, p-value < 0.001). There was no association of GBS colonization with the health care facility tier, pregnancy trimester or other demographic or clinical variables (Table 5).

Discussion

Streptococcus agalactiae has its natural reservoir in the human gastrointestinal tract, which serves as an important source of vaginal colonization in women. Colonization of the human rectovaginal tract with GBS is a risk factor for chorioamnionitis and transmission of the organism to the newborns [1, 22, 23]. In the present study, the overall proportion of *S. agalactiae* colonization was 24.5%, which is comparable to other reports from Africa (19.3–22.4%) [3, 4], but relatively higher than the prevalence of 7.0–11.0% from Asian countries and 12.7–19.0% from developed world [11, 24, 25]. Similarly, high prevalence was reported from country-specific studies in Africa such as 30.9–37.0% in South Africa [26, 27];

Table 5 Multivariable logistic regression analysis of the factors associated with GBS colonization in pregnant women

Characteristics	GBS culture positive, n (%)	Crude OR (95% CI)	p-value	Multivariable OR (95% CI)	p-value
Health care facility tier					
Lower (559)	423(75.7)	1.0			
Higher (313)	235 (75.1)	1.03 [0.75–1.42]	0.846		
Referral status					
Not referred (678)	174 (25.7)	1.0			
Referred (194)	40 (20.6)	0.75 [0.51–1.11]	0.151	0.90[0.54–1.50]	0.697
Marital status					
Married (667)	165 (24.7)	1.0			
Single (205)	49 (23.9)	0.96 [0.66–1.38]	0.808		
Residence					
Outside City (173)	36 (20.8)	1.0			
City (699)	178 (25.5)	0.77 [0.51–1.15]	0.204	1.44[0.93–2.23]	0.097
Education level					
Informal (58)	13 (22.4)	1.0			
Primary (428)	105 (24.5)	1.13 [0.54–2.17]	0.724		
Secondary and above (386)	96 (24.9)	1.15 [0.59–2.21]	0.685		
Parity					
Nullipara (0) (313)	81 (25.9)	1.0			
Multipara (1–4) (509)	123 (24.2)	0.91[0.66–1.26]	0.581		
Grandmultipara (≥) (50)	10 (20.0)	0.72[0.34–50]	0.375		
Gestation age					
1st trimester (28)	5 (17.9)	1.0			
2nd trimester (318)	87 (27.4)	1.7 [0.6–4.7]	0.280		
3rd trimester (526)	122 (23.2)	1.4 [0.5–3.7]	0.514		
HIV status					
Negative (761)	144 (18.9)	1.0			
Positive (111)	70 (63.1)	7.31[4.78–11.20]	< 0.001	7.33[4.77–11.29]	< 0.001
Current admission					
No (735)	187 (25.4)	1.0			
Yes (137)	27 (19.7)	0.72 [0.46–1.13]	0.154	0.94[0.53–1.69]	0.847
History of antibiotic use					
No (779)	193 (24.8)	1.0			
Yes (93)	21 (22.6)	0.89 [0.53–1.48]	0.642	0.99[0.57–1.72]	0.969
History of abortion					
No (712)	171(24.0)	1.0			
Yes (160)	43 (26.9)	1.16[0.79–1.72]	0.448		

and 31.6% in Gambia [5]. In our study, GBS-prevalence was significantly higher in HIV-positive women than in HIV-negative women. Thus, the difference in the GBS prevalence between Tanzania and South Africa could putatively be attributed to differences in the underlying HIV prevalence among pregnant women in the antenatal clinics (30.0% in the National Survey in 2019 in South Africa versus 5.6% in Tanzania) [28, 29]. However, HIV prevalence among pregnant women in Gambia from the National Sentinel Surveillance was as low as 1.82% [30], even though GBS prevalence in Gambia was similar to that in South Africa and higher than in Tanzania. Therefore, varying HIV burden across countries and regions should be taken into account together with other socio-cultural, epidemiological and clinical factors to address

country/regional specific mitigation strategies against GBS colonization and attributable adverse maternal fetal outcomes. Our study has shown a significant increase in the odds of GBS colonization among HIV infected pregnant women (63.1% vs. 18.9%, OR=7.33), similar to a study in the DRC (50% vs. 19.1%, OR=4.22) [14], but in other studies such differences were not statistically significant. For example in Malawi (44% vs. 35%, respectively) [6], in Ethiopia (24% vs. 13.7%) [13], and Brazil (19.8% vs. 14.1%) [31] prevalence was numerically higher but not statistically significantly higher in HIV-positive women. A study in Kenya paradoxically showed low prevalence of GBS colonization among HIV-positive women as compared with HIV-uninfected women [22]. The possible reasons for increase in GBS colonization among HIV

infected pregnant women may be related to their immunosuppressed state which increase their susceptibility to colonization and potentially subsequent infection with opportunistic pathogens (GBS inclusive). The correlation of GBS colonization with CD4+ T cell count and/or HIV viral load remains to be further explored in future studies as these variables were either not determined or not recorded in the hospitals involved, and therefore, there was insufficient data for meaningful analysis. In the USA, GBS prevalence has been linked to sexual activity, behavioral issues such as recent sexual activity, more than one sexual partner in the past 30 days, younger age and sexually transmitted infection (STI) clinics' attendees, similar to HIV infection and transmission [32]. Thus, the association between HIV and GBS status may be driven by shared risk factors. Regardless of the underlying mechanism, the preponderance of GBS colonization among HIV infected pregnant women calls for priority screening of this vulnerable group in Mwanza, Tanzania, and other areas throughout the country with similar epidemiological predisposition. In addition, re-focusing of research priorities to get more information on the cost-effectiveness of re-packaging of GBS screening with screening for other STI among pregnant women attending ANC is new area for evaluation.

Findings from our current study can be used to inform approaches to screening of pregnant women, including HIV-positive women, in our area. Firstly, the use of two swabs (rectal and vaginal swabs) is preferred to maximize recovery but if resource constraints necessitate the use of a single swab, rectal swabs should be prioritized, and secondly, GBS recovery from bacteriological media was significantly higher in CHROMagar™ Strep B agar than conventional blood agar, where feasible, CHROMagar™ Strep B agar can be used to increase recovery. This media is technically simple, time saving, and has higher sensitivity compared to technically demanding and time-consuming conventional methods like blood agar and microscopy. Similar high diagnostic utilities for CHROMagar™ Strep B agar were previously reported in India and Australia [20, 33]. However, the cost for CHROMagar™ Strep B agar is a stumbling block, and in settings (or when screening individuals) with economic challenges, even blood agar can still yield useful results when used following the standard laboratory operating procedures. To the best of our knowledge, this is the first study to report GBS colonization using CHROMagar™ Strep agar in a large sample size of pregnant women spanning from primary to a tertiary level health care facilities in East Africa.

In the previous two studies done in Mwanza and Dar es Salaam, Tanzania, GBS colonization among pregnant women in the third trimester in the two tertiary hospitals were 9.5% and 23%, respectively [9, 15]. In the

current study, GBS prevalence in Mwanza was similar to that reported from Dar es Salaam and much higher than previously reported from Mwanza. The increase in the proportion of GBS colonization in Mwanza from 9.5 to 24.5% may be attributable, at least in part, to the use of the CHROMagar™ StrepB agar which has higher bacterial recovery potential compared to the conventional media [16, 20]. It is not, however, linked to the inclusion of lower tier health care facilities in our current study because it was evident that GBS colonization was found in all health care facilities (ranging from 18.6 to 28.0%), with no significant difference between lower and higher tiers. Considering that even on blood agar, prevalence in the current study was much higher than in the previous study (18.8% vs. 9.5%), our findings highlight, at least in part, a true increase in the prevalence of GBS colonization in this region over time. Therefore, a need to introduce routine screening of GBS among pregnant women in the ANC irrespective of the health care facility tier is reiterated. The findings further shed light into further technical experts' discussion to re-consider introduction of highly sensitive GBS screening methods to prevent adverse maternal or fetal outcomes associated with this pathogen, and complement existing data on the role of gram-negative bacteria in Tanzania which has been widely delineated [34, 35].

The most efficient management option available at present in GBS colonized patients is intrapartum chemoprophylaxis and treatment with parenteral antibiotics. Based on CDC guidelines, GBS isolates are susceptible to penicillin and ampicillin antibiotics which are the first line drug of choice for intrapartum prophylaxis, while erythromycin and clindamycin are second line drugs in the prevention of early onset GBS neonatal infection [36]. In the present study, appealing results for GBS sensitivities were shown for ampicillin (100%), clindamycin (84.4%), and erythromycin (76.3%). Therefore, these agents can be judiciously used for GBS prophylaxis and treatment. Similar antimicrobial susceptibility profiles to ampicillin/penicillin and macrolides were shown from South Africa [37], Ethiopia [13], Saudi Arabia [38], US and UK [39, 40]. In contrast to these, alarmingly low prevalences of susceptibility of GBS strains were reported in China for both erythromycin (14.3%) and clindamycin resistance (26.8%) [41], highlighting a critical need to generate country-specific antibiograms to guide specific antimicrobial therapies.

Study limitations

This study was cross-sectional in nature, and therefore did not determine causal inferences or the outcome of GBS colonization to pregnant women and newborns. Many clients did not have CD4 and viral load records, and therefore sub-analysis of these variables was not

done. Prospective future studies measuring these variables in real time are recommended so to gain more insight into their significance. Molecular characterization of GBS isolates was not done due to financial constraints, however, all isolates were archived for future characterization. Finally, we did not explore on the influence of ethnicity on GBS colonization as all enrolled pregnant women were African in origin, and we propose evaluation of the association of tribes with GBS colonization in future studies.

Conclusions

Approximately a quarter (24.5%) of pregnant women are colonized by GBS in Mwanza, Tanzania with significantly higher colonization among HIV infected women. The GBS colonization is neither associated with trimesters nor health care facility tier, supporting a need to cascade screening services in all tiers of health care referral system. Ampicillin can be judiciously used for the prophylaxis and for treatment of GBS. Furthermore, for optimized GBS detection purposes, both vaginal and rectal swabs can be taken to increase GBS isolation. Introduction of routine GBS screening among all HIV-infected pregnant women using CHROMagarTMStrepB agar is recommended to complement existing maternal and neonatal screening for gram-negative bacterial colonization and infections. Molecular characterization of GBS isolates would be of interest to delineate circulating clones, linking them with antimicrobial susceptibility profiles and ultimately guiding future vaccination strategies.

Abbreviations

ANC	Antenatal Clinic
AST	Antimicrobial susceptibility testing
BMC	Bugando Medical Centre
CD4	Cluster of Differentiation 4
CI	Confidence Interval
GBS	Group B Streptococcus
HIV	Human Immunodeficiency Virus
KHC	Karume Health Centre
MHC	Makongoro Health Centre
MNH	Muhimbili National Hospital
NDH	Nyamagana District Hospital
OR	odds ratio
SDDH	Sengerema District Designated Hospital

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Author contributions

PHN, RK, SEM and JS: conceived, designed and executed the study. RK, NM, CD, SEM, RNZ and JS: Supervised, coordinated and provided technical, material and financial support to the study. PHN, BAR, STM, DM and FM: participated in data and sample collections. PHN, BAR, STM, and JS performed

microbiological laboratory procedures. PHN, RK, DM and NM participated in patients' management. PHN, RK, NM, DM, FM, CD, SEM, RNZ and JS: performed data analysis, literature search and interpretation of data. PHN prepared the first draft of the manuscript, which was critically reviewed by all authors. All authors have read and approved the final manuscript.

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Data availability

The dataset used and/or analyzed during the current study is available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

The Catholic University of Health and Allied Sciences and Bugando Medical Centre review board cleared the study (CREC/459/2021) and informed consent was obtained from participants. Participants were informed in detail about the study procedures, and were asked to sign a consent form if they were willing to participate in the study. For illiterate participants, information was read aloud and a thumbprint was requested for those who consented. For participants under 18 years old, apart from assenting to participate, their respective parents or guardians also consented for participation in this study.

Competing interests

The authors declare no competing interests.

Author details

¹Department of Obstetrics and Gynecology, Weill School of Medicine, Catholic University of Health and Allied Sciences & Bugando Medical Centre, Mwanza, Tanzania

²Department of Microbiology and Immunology, Weill-Bugando School of Medicine, Catholic University of Health and Allied Sciences, Mwanza, Tanzania

³National Public Health Laboratory, Ministry of Health, Dar Es Salaam, Tanzania

⁴Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, Allschwil, Switzerland

⁵University of Basel, Basel, Switzerland

⁶Sydney School of Veterinary Science, Faculty of Science, University of Sydney, Sydney, Australia

⁷Sydney Infectious Diseases Institute, Faculty of Medicine and Health, University of Sydney, Sydney, Australia

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