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Maternal vaginorectal colonization by Group B Streptococcus and *Listeria monocytogenes* and its risk factors among pregnant women attending tertiary hospital in Mwanza, Tanzania

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Abstract

Background: Group B streptococcus (GBS) and *Listeria monocytogenes* are members of the normal microbes of the female genital tract. During labour GBS and *Listeria monocytogenes* may infect the new-borns, leading to neonatal sepsis and meningitis. So far, there is no report on prevalence of GBS and *Listeria monocytogenes* among pregnant women in Mwanza. The objective of the study was to determine the magnitude of Group B Streptococcus and *Listeria monocytogenes* and its associated factors at Bugando Medical Centre, Mwanza, Tanzania.

Methods: The study was a cross section conducted from 1st November 2011 to 31st May 2012 at Bugando Medical Centre in Mwanza, Tanzania. Vaginal and rectal swabs were obtained and cultured on 5% sheep blood agar and susceptibility testing done using disk diffusion technique.

Results: A total of 295 pregnant women participated in the study. GBS strains were isolated from 28 (9.49%) and only two (0.68%) had isolates of *Listeria* spp. All GBS and *Listeria* spp. isolates were sensitive to penicillin and ampicillin. Eight GBS isolates were resistant to erythromycin (28.6%), seven GBS isolates were resistant to clindamycin (25%) and 15 of GBS isolates were resistant to tetracycline (53.6%). One *Listeria* spp isolate was resistant to cotrimoxazole. Pregnant women with no formal education and those dwelling in rural areas were more frequently colonized by GBS.

Conclusion: There is a significant prevalence rate of GBS culture positive at Bugando Medical Centre with demonstrable resistant to some common antibiotics (tetracycline, erythromycin and Clindamycin). Screening for GBS should be instituted in Tanzania between 35 and 37 weeks of gestation coupled with regular check up for antimicrobial susceptibility pattern due to emerging resistance toward existing antibiotics.

Keywords: Streptococcus, *Listeria monocytogenes*, pregnancy, neonatal sepsis, mortality, Tanzania

Introduction

Group B Streptococcus (*Streptococci agalactiae*) and *Listeria monocytogenes* are bacteria commonly found in the gastrointestinal tract (Lennon *et al.*, 1984; NCID, 2002). While GBS is present in up to one-third of the women of child bearing age in reproductive tract, *L. monocytogenes* is present in up to 15% of intestinal tract of health adult (Lennon *et al.* 1984; Artz *et al.* 2003). Globally approximately 10-30% of pregnant women are carrier for GBS (Moyo *et al.* 2002). In pregnant women, GBS is associated with an increased risk of infection such as bacteraemia, chorioamnionitis, endocarditis, urinary tract infections and arthritis while *Listeria monocytogenes* causes a mild influenza like illness (Gellin & Broome, 1989; Sendi *et al.* 2009).

Asymptomatic carriers of GBS are at an increased risk of adverse pregnancy outcome such as premature rupture of membrane, preterm delivery and low birth weight while infection with *Listeria monocytogenes* can lead to abortion, stillbirth, or delivery of an acutely ill infant (Zhu *et al.*, 2005; Dzowela *et al.*, 2006). Though GBS has the ability to cross through the intact amniotic membrane causing infection to the foetus but its clinical importance lies on the fact that it can be

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transferred to the neonate as it passes through the birth canal and cause serious infections with high mortality rate (Dzowela *et al.,* 2006). More than 98% of cases of early onset GBS sepsis are the consequence of vertical transmission from the genital tract of the mother to the foetus (Remington *et al.,* 2011). Treatment of GBS and *Listeria* infections during pregnancy can prevent transmission to the foetus (Kaistone, 1991; Schrag *et al.*, 2002).

While several parts of the world has adopted screening for GBS to all pregnant women near term, Tanzania is yet to start the programme despite a high rate of neonatal death related to vertical transmission of GBS and *Listeria monocytogenes* (Joachim *et al*.,2009;Kayange *et al*.,2010). There is paucity of data regarding the colonization rate of these bacteria among pregnant women in Tanzania despite isolation of the pathogens in neonates with sepsis (Kayange *et al.*, 2010). This study was therefore carried out determine The objective of the study was to determine the magnitude of Group B Streptococcus and *Listeria monocytogenes* and its associated factors at Bugando Medical Centre, Mwanza, Tanzania.

Materials and Methods

Study area *and population*

This cross sectional study was carried out at Bugando Medical Centre (BMC) in Mwanza, Tanzania from 1st November 2011 to 31st May 2012. BMC is a consultant and teaching hospital for the Lake and Western zones of United Republic of Tanzania. BMC is situated along the shores of Lake Victoria in Mwanza city and has a 900 bed capacity. It serves catchments population of approximately 13 million people. Pregnant women of different ages and socioeconomic status attending antenatal clinic at any gestation age between 28-42 weeks were included in the study. Pregnant women who have received antibiotic treatment within 2 weeks before recruitment were excluded from the study and so were those with critical conditions such that they were not be able to communicate. The sample size was estimated using Kish Leslie(Kish, 1965) formula for cross-sectional studies. The prevalence of colonization of 23% was used (Joachim *et al*. 2009).

Data collection

A pre-tested, structured questionnaire was used to collect the data. The questionnaire contained questions on respondent's socio-demographic characteristics, obstetrical history and other biodata. These included maternal age, residence, gestation age, parity, previous pregnancy outcome, HIV status, contraceptive use, occupation, diabetes mellitus and consumption of locally processed milk. Low vaginal swabs and perianal swabs were taken. Briefly the vaginal swab was taken without a speculum, by inserting the swab 2–3 cm into the vagina and rotating the swab with a circular motion, leaving it in the vagina for approximately five seconds. A separate perianal swab was taken by gently rotating the swab around the anal margin for approximately five seconds. Swabs were transported to the laboratory for processing according to standard operating procedures. The results of both screening swabs were made available to the caregivers.

Laboratory procedures

At the laboratory samples were inoculated directly and cultured on 5% sheep blood agar plates. Culture plates were then incubated at 37°C with 5% candle jars CO_2 for 24 hours. All suspected GBS colonies (beta-haemolytic, Gram- positive, catalase negative) and *Listeria* spp. were sub-cultured on 5% sheep blood agar for pure culture. For GBS presumptive identification was performed using SXT and Bacitracin (BA) disc. All isolates resistant to 0.05 unit of BA and resistant to 25µg of cotrimoxazole (SXT) were presumptive identified as GBS (Murray, 1995). These isolates were confirmed using CAMP test and positive agglutination with GBS Latex agglutination Kit (Oxoid UK). Susceptibility to various antimicrobial agents was tested using the disk diffusion technique. The antibiotics tested were ampicillin, penicillin, erythromycin,

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tetracycline and clindamycin. Heavy suspension of colonies prepared on normal saline was inoculated on 5% sheep blood agar using cotton swabs. Paper disks containing antimicrobial agents were placed on the plate. Plates were incubated for 24 hours in carbon dioxide atmosphere at 37°C. Zones of inhibition were measured and the results were interpreted according to Clinical Laboratory Standard Institute (CLSI, 2006).

Data analysis

Data were checked daily for completeness, cleared, edited, coded and double entered into the study computer database (Microsoft Excels) and then were exported to STATA version 10.1 (Texas 77845 USA) for analysis. Means, median, range and standard deviation were calculated for continuous variables. Frequencies and percentages were calculated for categorical variables. The categorical variables were analysed using Chi-square test or Fischer's exact. $P<0.05$ was considered significant. Odds ratios (OR) were the measure of association and confidence intervals (95% CI) were reported.

Ethical considerations

The study was approved by Catholic University of Health and Allied Sciences/Bugando Medical Centre Ethics Review Board. Informed consent from participants was sought before being recruited in the study. Confidentiality of the study subjects was ensured throughout the study. Prophylactic antibiotics (intramuscular penicillin 5MU stat then 2.5MU 4 hourly until delivery) were prescribed to pregnant women with GBS and/or *Listeria spp* culture positive results when they had ruptured membrane or were in labour.

Results

Socio-demographic characteristics of the participants

A total of 295 participants were recruited, age ranging between 14 and 39 years with mean of 25.6 ± 0.31 years. The gestation age of the participants ranged from 28 weeks to 42 weeks with a mean gestation age of 34.6 ± 0.23 weeks. Majority of the respondents were married 87.85% and reside in urban area 62.7%. Additionally, 64.4% of respondent had no formal employment and about 61% had primary level of education (Table1).

Table 1: Baseline characteristics of the 295 pregnant women from Bugando Medical Centre

Variable	Response	Total (N=295)	Percentage
Marital status	Living alone	36	12.2
	Living with a partner	259	87.8
Residence	Rural	110	37.3
	Urban	185	62.7
Occupation	Employed	105	35.6
	Unemployed	190	64.4
Education	No formal education	28	9.5
	Primary	181	61.4
	Secondary	73	24.7
	Tertiary	13	4.4
Unpasturalised milk	Yes	112	38
	No	183	62
HIV status	Positive	9	3.0
	Negative	286	97.0

Prevalence and colonization of GBS and Listeria spp

The overall prevalence of GBS colonization was 9.49% and that of *Listeria* spp was 0.68%. Of the 28 patients carrying GBS, isolates were cultured from only vaginal swabs in 13 cases (46.4%), only from rectal swabs in 10 cases (35.7%), from both vaginal and rectal swabs in five cases (17.9%).

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Women more than 25 years had higher rate of GBS colonization (9.94%) but not significant {OR 1.12, 95% CI (0.51-2.46), p=0.774)}. GBS colonization tends to decrease as the level of education increase. Those with no education were significantly more colonized than those with higher education {OR 12.1 95% CI (2.29-64.51), p=0.003}. Pregnant women from in rural areas were more colonized by GBS than those from urban areas but not statistically significant {OR 1.29, 95% CI (0.59-2.85) p=0.528}. Marital status, occupation and use of contraceptive were not associated with GBS colonization (Table 2) .

Table 2: GBS colonization by demographic characteristics

Characteristics	Response	GBS negative (n=267)	GBS carrier (n=28)	OR (95%CI)	P value
Age	<25 years	122(91.04%)	12(8.96%)	1.0	
	≥25 years	145(90.06%)	16(9.94%)	1.12(0.51-2.46)	0.774
Marital status	Living alone	33(91.7%)	3(8.3%)	0.85(0.24-2.97)	0.800
	Living with partner	234(90.4%)	25(9.7%)	1.0	
Education	No school	21(77.8%)	6(22.2%)	12.1(2.29-64.51)	0.003
	Primary	161(89.0%)	20(11.0%)	5.3(1.21-23.12)	0.027
	Secondary	85(97.7%)	2(2.3%)	1.0	
Occupation	Employed	96(91.4%)	9(8.6%)	1.0	
	Unemployed	171(90.0%)	19(10.0%)	1.18(0.51-2.72)	0.689
Residence	Urban	169(91.3%)	16(8.7%)	1.0	
	Rural	98(89.1%)	12(10.9%)	1.29(0.59-2.85)	0.523

GBS and Listeria spp colonization by obstetric characteristics, diseases and food intake

Pregnant women with bad previous perinatal outcome were more colonized but not statistically significant {OR 2.0, 95% CI (0.76-5.31), p=0.163}. Parity, abortion and gestation age were not associated with GBS colonization (Table 3). *Listeria spp* were isolated from multigravida pregnant. Parity, history of abortion and bad pregnancy outcome were not associated with *Listeria spp* colonization. Neither GBS nor *Listeria spp* colonization was associated with HIV status or diabetes mellitus. Of the 295 pregnant women recruited, nine (3 %) were HIV positive and two (0.75%) had diabetes mellitus but none of them had GBS colonization. Similarly, none of pregnant women with either HIV or diabetes mellitus was colonized by *Listeria spp.* Drinking unpasturalized milk in our study was not associated with *Listeria spp* colonization. None of 112 pregnant women who drank locally processed milk was colonized by *Listeria* spp.

Table 3: GBS colonization by obstetric characteristics and diseases

Characteristics	Response	GBS negative (n=267)	GBS carrier (n=28)	OR (95%CI)	P value
Gravidity	Primigravida	78(90.7%)	8(9.3%)	1.0	
	Multigravida	189(90.4%)	20(9.6%)	1.03(0.44-2.44)	0.943
Parity	Nullipara	90(90.0%)	10(10.0%)	1.5(0.51-4.42)	0.454
	Primipara	65(92.9%)	5(7.1%)	1.0	
	Multipara	112(89.6%)	13(10.4%)	1.4(0.47-4.43)	0.502
Gestation age	<37weeks	165(90.7%)	17(10.3%)	1.0	
	≥37weeks	102(90.3%)	11(9.7%)	1.05(0.47-2.52)	0.911
Abortions	No history	229(90.5%)	24(9.5%)	1.0	
	At least one	38(90.5%)	4(9.5%)	1.00(0.33-3.06)	0.994
History of bad neonatal outcome	No	235(91.4%)	22(8.6%)	1.0	
	Yes	32(84.2%)	6(15.8%)	2.0(0.76-5.31)	0.163
HIV status	Negative	258(90.2%)	28(9.8%)	1.000*	
	Positive	9(100%)	0(0.0%)		
Diabetes mellitus	No	265(90.5%)	28(9.5%)	1.000*	
	Yes	2(100%)	0(0.0%)		

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Antimicrobial susceptibility pattern of GBS and Listeria spp

All 28 GBS isolates were susceptible to Penicillin and Ampicillin. Fifteen GBS isolates (53.6 %) were resistant to tetracycline, eight (28.6%) of them showed resistance toward Erythromycin and seven (25%) were resistant to clindamycin (Table 4).

Table 4: Susceptibility pattern of 28 GBS isolates

Isolate	Penicillin	Ampicillin	Erythromycin	Tetracycline	Clindamycin
V-32⁺	S	S	R	R	S
Rt-36	S	S	S	S	S
V-55	S	S	S	R	S
V-93	S	S	S	R	R
Rt-93	S	S	S	S	S
V-96	S	S	S	R	S
Rt-109	S	S	R	S	R
Rt-116⁺	S	S	R	R	R
V-118	S	S	S	S	S
Rt-118	S	S	S	S	S
V-124⁺	S	S	R	R	R
V-127	S	S	S	S	S
V-133	S	S	R	S	S
Rt-143	S	S	S	R	S
Rt-172	S	S	S	R	S
V-182	S	S	S	S	S
Rt-182	S	S	S	S	S
V-188	S	S	S	R	S
Rt-189	S	S	S	R	S
V-190	S	S	S	S	S
Rt-202	S	S	R	R	R
V-235	S	S	S	S	S
Rt-235	S	S	S	S	S
V-240	S	S	S	S	S
Rt-249	S	S	R	R	S
Rt-266	S	S	S	S	R
Rt-273	S	S	S	R	S
V-278	S	S	S	R	S
V-281⁺	S	S	R	R	R
Rt-294	S	S	S	S	S

⁺=D Test positive (erythromycin induced clindamycin resistance); V= vaginal swabs; Rt=rectal swabs; S=Sensitive, R=resistant

Discussion

The prevalence rate of positive GBS culture in our study population was almost similar to findings of a study in Zimbabwe (Moyo *et al.* 2002) but was lower compared to that reported by Joachim *et al.* (2009) in Dar es Salaam, Tanzania. This might be due to difference on culture media. In our study a non-selective blood agar was used as compared to selective culture media used by Joachim *et al.* (2009). A similar lower prevalence rate in pregnant women using similar non-selective blood agar has been reported by Thinkhamrop *et al.* (2003). The recommended selective media of choice for GBS colonization is Todd-Hewitt media (Schrag *et al.* 2002). However some studies reported similar prevalence rate of GBS colonization using Todd-Hewitt media and non-selective media (blood agar) (Werawatakul *et al.* 2001). Despite these differences in technique, region variations exist. For example, GBS colonization is highly prevalent in Zimbabwe, Gambia and Nigeria (Baker & Barrett, 1973; Moyo *et al.*, 2002; Dziweka *et al.*, 2006). Low prevalence rates have also been reported in Mozambique, Ethiopia and Togo (Baker & Barrett, 1973).

Comparing rectal against vaginal samples, this study found that the detection rate of GBS was high in vaginal than rectal sample. Philipson *et al.* (1995) and Tsui *et al.* (2009) reported

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similar findings with relatively high proportion than in our study. Perhaps the use of Todd-Hewitt culture media could explain the higher rates reported by these studies (Bosch-Mestres *et al*. 2003). There is little information regarding differences between pregnant women with rectal versus vaginal colonization. However Meyn *et al.* (2002) noted a significant rate of vaginal colonization with increased recent sexual activity. Since majority of our carriers had vaginal colonization, it may be worth investigating their sexual practices during pregnancy.

This study has shown that there is no association between HIV infection and GBS colonization among pregnant women. Similar observations have been reported from other studies elsewhere (El Beitune *et al.,* 2006; Shah *et al.,* 2011). This indicates that HIV infection is not a risk factor for GBS colonization among pregnant population attending the Bugando Medical Centre in Mwanza.

In this study we found that pregnant women living in rural areas were more frequently colonized with GBS than their counterparts from urban areas though this association was not statistically significant. In Zimbabwe, Mavenyengwa *et al.* (2010) reported a significant higher rate of colonization in the rural areas than in the urban areas. Probably the difference in hygiene practices between the two groups of people could explain these variations in the rate of colonization. In this study, GBS colonization was not affected by host risk factors. Inconsistent findings have been observed in different studies (Baker *et al.*, 1975; Papapetropoulou & Kondakis, 1987; Anthony *et al.*, 1978; Valkenburg-Van den Berg *et al*., 2006). Some studies have reported an association between age and gravidity and GBS colonization but not others (Anthony et al., 1978; Yow et al., 1980; Papapetropoulou & Kondakis, 1987). However in our study we found a significant high rate of colonization by GBS in uneducated pregnant women. Perhaps this sub population with no formal education had difficult in adhere to hygiene practices than those with education.

As observed in other part of the world high carriage of erythromycin and clindamycin resistant GBS was observed in this study (DiPersio & DiPersion, 2006; Janapatla *et al.,* 2008). This can be due to over prescription of these drugs especially erythromycin. The results alert of the possibility of inadequate prophylaxis when using these antimicrobial drugs as an alternative in penicillin allergic patients.

In most studies, maternal GBS screening was performed at term between 35 to 37 weeks as such culture results would be more representative of colonization status at delivery (Yancey *et al.,* 1994). The mean gestation age at study entry in this study was 34.6 ±0.23 weeks. Therefore swabs taken at study entry in served the purpose since the aim was to determine the prevalence of GBS carriage and associated risk factors. In this population, a cohort study of carrier status at delivery would have been of interest, especially if correlated with neonatal outcome.

With regard to *L. monocytogenes,* the prevalence was found to be low. This low prevalence rate is consistent with findings of a study among women of reproductive age in Serbia (Stepanović *et al*. (2007). A study by Borges *et al.* (2011) has shown that the bacteria are inhibited by the normal vaginal pH, and this is likely to have attributed to its low prevalence in our study subjects. Some previous studies suggested an association between chronic carriage of *Listeria* and recurrent abortion, but this has not been a consistent finding (Macnaughton, 1962). In our study one pregnant woman with *Listeria spp* isolate had history of at least one abortion but it was not statistically significant to conclude the association. Similarly in one prospective study, *Listeria* was not isolated from the cervix or the endometrium of any of the 86 patients with two or more foetal losses (Manganiello & Yearke, 1991). Furthermore, we could not establish any association between host risk factors such as diabetes mellitus and HIV status and *Listeria spp* colonization. This is likely to have been contributed to the small sample size with diabetes mellitus and HIV. About one-third of the study participants reported the use of unpasturalized milk during their current pregnancy but none was found with infected with *Listeria spp* isolate. Epidemiological investigations have demonstrated that nearly all types of food can transmit *Listeria spp* (Janakiraman, 2008). Thus, establishing guidelines that will prevent exposure to *Listeria spp* is

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likely to be inappropriate. Obstetricians therefore, should provide dietary advice to their clients as recommended by Centers for Disease Control and Prevention to reduce the risk (Janakiraman, 2008).

Penicillin, ampicillin, and amoxicillin have been used frequently in the treatment of listeriosis (Temple & Nahata, 2000). These drugs block several penicillin-binding proteins and do penetrate intracellular. Microbial resistance of *Listeria* to penicillin or its derivatives has not yet been found under natural conditions (Hof *et al*., 1997). Similarly in our study both Listeria isolates were sensitive to ampicillin and penicillin with one isolate being resistant to cotrimoxazole.

In conclusion, there is a significant prevalence rate of GBS culture positive among patients attending Bugando Medical Centre in Mwanza with demonstrable resistant to some common antibiotics. Screening for GBS infection should be instituted in Tanzania between 35 and 37 weeks of gestation. A regular check up for antimicrobial susceptibility pattern should be mandatory due to emerging resistance toward existing antibiotics.

Competing of interests

The authors declare that they have no competing interests.

Author's contributions

AE participated in proposal development, data collection, data analysis and manuscript writing, NN participated in proposal development and data analysis, EN participated in manuscript writing, AM participated in manuscript writing, AK participated in data analysis, SD participated in manuscript writing and SM participated in proposal development, processing and interpretation of swabs, data analysis and manuscript writing.

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