EAHRC
EAST AFRICAN HEALTH RESEARCH COMMISSION
Research for Health and Prosperity

ORIGINAL ARTICLE

Multi-drug resistant facultative pathogenic bacteria colonizing the vagina of pregnant women with premature rupture of membrane, Tanzania

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ABSTRACT

Background: Premature rupture of membrane (PROM) contributes to approximately one-third of premature birth and 10% perinatal mortality worldwide. Here, we report the patterns of facultative pathogenic bacteria colonizing the vagina of pregnant women to guide prophylactic antibiotic treatment in the management of PROM.
Methods: This comparative cross-sectional study was conducted between August 2015 and March 2016. High vaginal swabs were collected and processed to detect the presence of facultative pathogenic bacteria. Isolate identification and antibiotic susceptibility testing was conducted using MALDI-TOF MS and VITEK-2 system, respectively. Data were analyzed using STATA version 13.
Results: A total of 175 pregnant women with PROM and 175 without PROM were investigated. The median age of the pregnant women with PROM was significantly higher than that of pregnant women without PROM: 27 [21-32] vs. 25[21-29], p=0.026. Pregnant women with PROM were significantly more likely to be colonized with facultative pathogenic bacteria 59/175 (33.7%), 95% CI; 26.7-40.7 than pregnant women without PROM; 27/175 (15.4%), 95% CI; 10.1-20.7, P<0.001. *Escherichia coli* were significantly more isolated from pregnant women with PROM than those without PROM: 36 (73.5%) vs. 13(26.5%), p<0.001. The proportion of resistance among pathogenic isolates from women with PROM to ampicillin, trimethoprim/sulfamethoxazole and cefotaxime were 100%, 66.7% and 40%, respectively.
Conclusions: The vagina of pregnant women with PROM is significantly more colonized by multi-resistant facultative pathogenic bacteria than that of pregnant women without PROM. Further studies should be done to elucidate the impact of these bacteria in relation to PROM and the pregnancy outcome.

Key words: Vaginal colonization, facultative pathogenic bacteria, pregnancy, premature rupture of membrane, multi drug resistance

INTRODUCTION

Worldwide, premature rupture of membranes (PROM) among pregnant women has been found to range from 3.3% to 10%, with 80% of them occurring at term[1,2]. PROM leads to the loss of the natural protection of the fetus hence posing a threat to bacterial infections[3]. In addition, PROM is highly associated with the increased pregnancy complications such as preterm labor, fetal demise, respiratory distress syndrome, neonatal sepsis, umbilical cord prolapse, postpartum endometritis, disseminated intravascular coagulopathy (DIC) and chorioamnionitis[1,4]. The pathogenesis of PROM has been linked to the isolation of facultative

pathogenic bacteria in the vagina[5-8], there is a strong association between pathogens colonizing the vagina and subsequent chorioamnionitis. Bacteria and protozoan parasites (*Trichomonas vaginalis*) secrete proteases and other factors that degrade the collagen and weaken the fetal membrane[9,10]. Furthermore, host inflammatory responses due to pathogenic bacteria can induce the production of prostaglandin which can lead to uterine irritability and membrane collagen degradation hence increasing the risk of PROM[8,11].

In developing countries, *Escherichia coli*, *Klebsiella pneumoniae*, Group B Streptococcus (GBS), *Staphylococcus aureus* and *Streptococcus pyogenes* have been found to be the commonest facultative pathogenic bacteria colonizing the vagina and implicated in PROM [5-7,12.] These pathogenic bacteria have also been implicated in chorioamnionitis[13,14]. Despite 12% contribution of the PROM to antenatal hospital admissions in East Africa[15] and black women being reported to have the highest risk of being colonized by the potential pathogenic bacteria[16], the spectrum of the respective bacteria colonizing the vagina of pregnant women with and without PROM in East Africa is not well understood.

A previous study[17] in Uganda among pregnant women with PROM, noted the resistance to most commonly used antibiotics such as ceftriaxone, ampicillin, trimethoprim/sulfamethoxazole and erythromycin was high with good susceptibility to expensive antibiotics such as vancomycin and meropenem. The World Health Organization (WHO) recommends the use of antibiotic prophylaxis among pregnant women with PROM[18]. Different regimen involving penicillin and erythromycin have been recommended. However, number of factors should be assessed such as population of women to be offered antenatal prophylactic antibiotics and types of antibiotics to be used. With increase antibiotic resistance among the pathogens involved in PROM local susceptibility data are crucial in establish empiric treatment protocol with emphasis on the individual tailored treatment. This comparative cross-sectional study was designed to investigate the patterns of facultative pathogenic bacteria colonizing pregnant women with and without PROM and their antimicrobial susceptibility.

METHODS

Study design, area, and population

The comparative cross-sectional study was conducted between August 2015 and March 2016 at Bugando Medical Centre (BMC), Sekou Toure Regional Hospital, Nyamagana District Hospital and Buzuruga Health Center in the city of Mwanza, Tanzania. The selected hospital are the highly populated faith base and government hospital which cover large percentage of the Mwanza population. Bugando Medical Centre is the tertiary consultant teaching hospital of Catholic University of Health and Allied Sciences with bed capacity of 900. Sekou Toure Regional Referral hospital is located in Ilemela district with bed capacity of 375 serving the all referrals from six district hospitals of Mwanza region. While Nyamagana District hospital has maternity bed capacity of 30 and Buzuruga health center has maternity ward with a bed capacity of 15. The study involved all pregnant women with gestation age of 36 weeks and above with and without PROM during the study period.

Sampling, inclusion, and exclusion criteria

Using Kirkwood formula for comparative studies[19] and assumption of the 10% effect size by the prevalence obtained in the previous study[12,] the minimum sample size obtained was 350 pregnant women (175 pregnant women with and 175 pregnant women without PROM). The pregnant women with and without PROM were recruited serially until the sample size was reached. The study excluded all pregnant women with cervical incompetency, polyhydramnios, mal-presentation, multiple pregnancies, fever, abdominal pain, foul smelling per vaginal leakage and history of antibiotic therapy in the past two weeks prior to the study to minimize the bias in relation to the colonization of the facultative bacteria. In the current study, pathogenic bacteria were defined as bacteria which are potentially capable of causing clinical infections in the genital urinary tract[20].

Sample collection and laboratory procedures:

By the use of sterile Cusco speculum, cervix was exposed, and high vaginal swab was taken using a sterile swab. The swabs were transported to the microbiology laboratory using Stuart transport media (HiMedia, India) within 2 hours of collection. All swabs were cultured on the 5% sheep blood agar (BA) and MacConkey agar (MCA) (Oxoid, UK) and aerobically incubated at 37ºC for 24-48 hours. Identification to species level was done by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (Bruker Daltonics, Germany) on extracted cells as previous described[21,22.] Only facultative pathogenic bacteria were included for statistical analysis and subsequent antimicrobial susceptibility testing. Potential contaminants, e.g. *S. epidermidis*, *Bacillus* spp., were excluded. The current study mainly concentrated on the antibiotics that are recommended for PROM management. The tested antibiotics included: ampicillin (AMP), trimethoprim/sulfamethoxazole, (SXT), ciprofloxacin (CIP), Gentamicin (CN), ceftazidime (CAZ), ceftriaxone

(CRO) and meropenem (MRP) (Oxoid, UK). Antimicrobial susceptibility testing was done using VITEK-2 system (bioMérieux, France) and interpreted as per EUCAST (http://www.eucast.org/clinical_breakpoints/) guidelines. Using excel sheet, data were double entered, cleaned and transferred to STATA version 11 for analysis. Categorical variables such as residence, marital status, education, occupation, gravidity, history of PROM, number of antenatal care (ANC) visit, presence or absence of PROM and positive or negative bacterial growth were summarized as proportions. Continuous data (age, gestation age and parity) were summarized using median and inter quartile range. The statistical significance was set at a *p* value of less than 0.05. Two-sample test of proportions was used to compare the pattern of facultative pathogenic bacteria colonization among pregnant women with PROM and those without PROM.

Ethics approval and consent to participate

The joint CUHAS/BMC research ethics and review committee granted ethical clearance with certificate number CREC/096/2015. Permission to conduct the study was sought from all hospital administrations. All patients were requested to sign a written informed consent before recruitment was done.

RESULTS

Demographic characteristic

A total of 350 pregnant women (175 with PROM and 175 without PROM) were enrolled and analyzed. The majority of studied women resided in urban areas 286(81.7%) and had primary school education 185 (52.9%). The median age of pregnant women was 26 [21-31] years. The median age of pregnant women with PROM was significantly higher than pregnant women without PROM 27 [21-32] vs. 25 [21-29], p=0.026 ***(Table 1)***.

The median gestation age of pregnant women at the time of enrollment was 38[36-40] weeks for pregnant women with PROM and 38[37-39] for pregnant women without PROM. History of PROM in previous pregnancy was higher among pregnant women with PROM than pregnant women without PROM 20 (11.4%) vs. 6 (3.4%), p=0.004. In addition, history of preterm birth in the previous pregnancy was higher among pregnant women with PROM than those without PROM 16(72.7%) vs. 6 (27.3%), p=0.026. Regarding antenatal visits, high proportion of pregnant women with PROM had more than 4 antenatal visits compared to pregnant women without PROM 92 (52.6%) vs. 54 (30.9%), p< 0.001 ***(Table 1)***.

Bacterial colonization pattern

Of 350 women screened, 86 (24.6%) were colonized with facultative pathogenic bacteria. Pregnant women with PROM were significantly more often colonized with facultative pathogenic bacteria 59/175 (33.7%), 95% CI; 26.7-40.7 than pregnant women without PROM 27/175 (15.4%), 95% CI; 10.1-20.7, p<0.001. The most frequently isolated bacteria were *Escherichia coli* 49(39.2%) and *Pseudomonas spp*. 22(17.6%). *E. coli* isolates were significantly more from pregnant women with PROM than those without PROM, 36 (73.5%) vs. 13 (26.5%), p<0.001 ***(Table 2)***. Of 175 pregnant women with PROM, 13(7.4%) had double colonization with two different species of facultative pathogenic bacteria. All pathogenic bacteria isolated from pregnant women with PROM were resistant to ampicillin, while those isolated from pregnant women without PROM were 92.6% (25/26) resistant to ampicillin. The proportion of resistant bacteria from women with PROM to ampicillin, trimethoprim/sulfamethoxazole and cefotaxime were 57(100%), 46(66.7%) and 28(40%), respectively while for women without PROM the proportion was 25(96.2%), 21(52.5%) and 20(48.8%), respectively ***Table 3***. *E. coli* isolates from pregnant women with PROM were significantly more resistant to trimethoprim/sulphamethoxazole than *E. coli* isolates from women without PROM 82.9% (29/35) vs. 58.3% (7/12), p<0.001 ***Figure 1***. One *E. coli* isolate from pregnant woman with PROM was resistant to all antibacterial agents tested (ampicillin, ciprofloxacin, gentamycin, trimethoprim/sulphamethoxazole, ceftriaxone, ceftazidime, cefotaxime, ertapenem, meropenem).

TABLE 1. ***Socio-demographic and clinical data of 350 pregnant women studied***

Patient characteristic	Total n=350 (%)	PROM n=175 (%)	Non-PROM n=175 (%)	*p* –value
Age* years	26 [21-31]	27 [21-32]	25 [21-29]	0.026
Residence				
Urban	286 (81.7)	131 (74.86)	155 (88.6)	
Rural	64 (18.3)	44 (25.14)	20 (11.4)	0.001
Marital status				
Single	73 (20.85)	46 (26.3)	27 (15.44)	
Married	277(79.15)	129 (73.7)	148 (84.57)	0.013
Education				
Primary	185(52.85)	91 (52)	89 (50.9)	
Secondary	123(35.14)	68 (38.9)	55 (31.4)	
University	42 (12)	16 (9.14)	31 (17.7)	0.071
Gravidity				
Prime	100(28.6)	36 (20.6)	64 (36.6)	
Gravid 2	175(50.0)	86 (49.0)	89 (50.9)	
Multigravida	75 (21.4)	53 (30.3)	22 (12.6)	<0.001
GA* weeks	38 [36-40]	38 [36-40]	38 [37-39]	0.012
PROM before				
No	324(92.6)	155 (88.6)	169 (96.6)	
Yes	26 (7.4)	20 (11.4)	6 (3.4)	0.004
GA at booking*	20 [18-22]	20 [18-22]	20 [18-22]	0.236
ANC visit				
Below 4	79 (22.6)	29 (16.6)	50 (28.6)	
4	125(35.7)	54 (30.9)	71 (40.6)	
Above 4	146(41.7)	92 (52.6)	54 (30.9)	<0.001

GA is gestation age, * Are variables were median is the measure of central tendency

TABLE 2. ***Vaginal pathogenic bacteria colonizing 350 pregnant women in Mwanza***

BACTERIA	PROM N (%)	Non-PROM N (%)	p- value
E. coli (49)	36 (73.5)	13 (26.5)	<0.001
Pseudomonas spp. (22)	12 (54.6)	10 (45.5)	0.273
K. pneumoniae (17)	11 (64.7)	6 (35.3)	0.043
Enterobacter spp. (13)	8(61.5)	5(38.5)	0.119
Pathogenic GPB* (10)	9(90)	1(10)	0.002
Acinetobacter spp. (7)	1(14.3)	6(85.7)	0.0038
Other GNB* (7)	5(71.4)	2(28.6)	0.054
Total (125)	82(65.6)	40(34.4)	<0.001

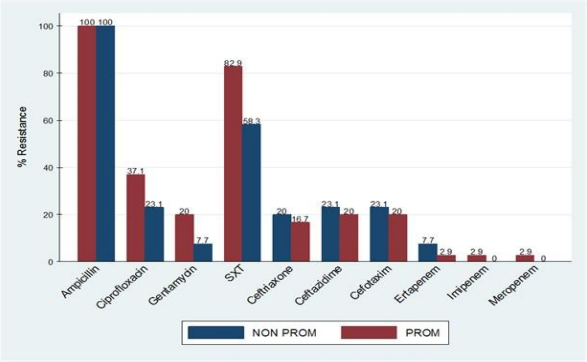
Pathogenic GPB stands for pathogenic gram-positive bacteria which include *S. aureus, S. haemolyticus, E. faecalis* and *S. saprophyticus* and other GNB stands for other gram-negative bacteria which include *Proteus* spp., *Morganella morganii* and *Escherichia hermannii.

TABLE 3. ***Antimicrobial resistance pattern among vagina pathogenic bacteria isolates***

	PROM		Non-PROM	
Antibiotic	**Tested**	**Resistant (%)**	**Tested**	**Resistant (%)**
Ampicillin	57	57 (100)	26	25 (96.2)
Ciprofloxacin	69	22 (31.9)	41	7 (17.1)
Gentamycin	69	16 (23.2)	41	8 (19.5)
SXT*	69	46 (66.7)	40	21 (52.5)
Ceftriaxone	57	14 (24.6)	22	3 (13.6)
Ceftazidime	70	17 (24.3)	40	9 (22.5)
Cefotaxim	70	28(40)	41	20 (48.8)
Ertapenem	57	1(1.8)	26	1 (4)
Imipenem	68	3(4.4)	41	0 (0.0)
Meropenem	68	2(2.9)	41	0 (0.0)

***SXT: trimethoprim/sulphamethaxazole**

FIGURE 1*: Antibacterial resistance pattern of E. coli colonizing vagina of pregnant women with and without premature rupture of membrane*



***SXT: trimethoprim/sulphamethaxazole**

DISCUSSION

Premature rupture of the membrane (PROM) contributes to approximately one third of premature births[2,23,24], and approximately 10% of perinatal mortality[25]. A significant proportion of pregnant women with PROM in the current study had history of PROM and premature delivery in the previous pregnancy. This has also been reported elsewhere[26, 27] and could be due to the possibility of the pregnant woman genetic defect in collagen synthesis that can affect the structure and function of the fibrillar collagens[28]. The weakening of the connective tissue by the enzymatic depolarization of the collagen fibers in fetal membrane can also explain the observed findings[29,30]. Women with PROM in the current study were significantly older than those without PROM pointing to the possibility of age-dependent collagen synthesis[31]. However, there is no documentation of the influence of age and facultative pathogenic bacteria colonizing vagina during the reproductive age. The age has been found to influence normal flora before puberty and after menopause[32, 33.]

Vaginal colonization with facultative pathogenic bacteria was significantly more often observed in pregnant women with PROM than in pregnant women without PROM. This has also been reported in previous studies in Tanzania and India[12,25]. Bacteria in the vagina have been found to secrete enzymes that can either degrade the fetal membranes or increase production of prostaglandins[4,12,34-39] high concentration of prostaglandins can stimulate the uterine contractions leading to the membrane rupture. This is further supported by the fact that the presence of pathogenic bacteria has been associated with chorion thinning among PROM pregnant women[40.]

As it was also previously reported in Tanzania and India[12,41], *E. coli* and *Pseudomonas* spp. were the commonest bacteria detected. This could partly be explained by the fact that these pathogens belong to the normal flora of the gastrointestinal tract and might therefore be present in perineum with increased chance to colonize the genital tract. *Staphylococcus aureus* was the commonest gram-positive bacterial species detected among pregnant women with PROM in this study. Similar observations were made previously[12 25]. Detection of *S. aureus* colonizing women with PROM has been linked with other factors like urinary tract infections and bacterial vaginosis[25], these factors were not investigated in the current study.

Bacteria isolated from pregnant women with PROM were more resistant to ampicillin and trimethoprim/sulfamethoxazole. Similar results have been reported in other studies from pregnant women[42,43] and post-delivery women in similar settings[44]. This could partly be explained by the fact that ampicillin and trimethoprim/sulfamethoxazole are the commonest class of antibiotic in use in the study settings. Despite the fact that this study has clearly demonstrated the significant differences in patterns of bacteria colonizing pregnant women with PROM and those without PROM, neonatal outcomes which could give more evidence on the association of vagina colonization of the facultative pathogenic bacteria and clinical fetal infections especially for the pregnant women with PROM were not recorded. Furthermore, the presence of *Candida* species were not assessed, this has been recommended for future studies.

In conclusion, vagina of pregnant women with PROM was more colonized by multi-resistant facultative pathogenic bacteria than the one of pregnant women without PROM. *Escherichia coli* strains were the commonest pathogenic bacteria and were highly resistant to ampicillin and trimethoprim/sulphamethoxazole. Further studies should be done to elucidate the impact of these pathogens in relation to PROM and the pregnancy outcome. There is a need to adjust the empirical prophylaxis treatment of PROM based on the local susceptibility profile.

***Acknowledgement*:** We thank member of the Department of Obstetrics and Gynecology, Microbiology and Immunology and Institute of Medical Microbiology Gottingen for their technical support. All authors have declared no conflict of interest in publishing this work.

Disclosure: None declared.

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Peer Reviewed

Competing Interests: None declared.

Received: 14 Jan 2020**; Accepted:** 3 Apr 2020.

Cite this article as: Kamgobe E, Grote S, Mushi MF, Wilson D, Gandye L, Bader O, Mshana SE, Groβ U. Multi-drug resistant facultative pathogenic bacteria colonizing vaginal of pregnant women with premature rupture of membrane, Tanzania. *E Afr Sci*. 2020;1(2):29-35. http://doi.org/10.24248/EASci-D-20-00001