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Contribution of vaginal infection to preterm premature rupture of membrane and adverse pregnancy outcome

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ABSTRACT

Background: Preterm premature rupture of membranes (PPROM) is the cause of approximately one third of preterm deliveries. **Objectives:** assess the relation of vaginal infections and their antimicrobial profile with PPRM and pregnancy outcome. **Methodology:** Case control study of 320 females with PPRM (case) and 320 females with normal pregnancy (control) at 28- 37 weeks of gestation. Vaginal examination, vaginal pH assessment and Whiff test were done. Vaginal swabs were collected and examined microscopically for diagnosis of different vaginal infections. Swabs were cultivated, identification and antimicrobial susceptibility of revealed bacteria were done. Maternal and neonatal outcomes were assessed. **Results:** Bacterial vaginosis and aerobic vaginitis were identified in 29.1% and 17.3% of all participants respectively. There was statistically significant difference regarding prevalence of different vaginal infections in case and control groups ($p < 0.001$). Aerobic vaginitis and bacterial vaginosis were risk factors for PPRM. *Streptococcus agalactiae* was the most prevalent organism. Erythromycin and ampicillin were the least effective antibiotics against Gram positive and Gram-negative isolates respectively. There was significant increase of all maternal and fetal adverse outcomes in cases with aerobic vaginitis. **Conclusion:** Different vaginal infections carry risk of PPRM and adverse maternal and neonatal outcomes. The variation in prevalence of bacterial isolates in different studies and localities notify the lack of standardized treatment for infected mothers. Accurate diagnosis of vaginal infection, precise medical treatment during pregnancy is essential for maintenance of maternal and neonatal health.

Introduction

Premature rupture of membranes (PROM) is defined as rupture of fetal membranes before onset of labor. Fetal membrane rupture occurring between 24 and 37 weeks is called preterm premature rupture of membranes (PPROM). Preterm PROM occurs in 3% of pregnancies and is the cause of approximately one third of preterm deliveries [1].

The etiology of PROM is multifactorial. Numerous risk factors are identified e.g. smoking, polyhydramnios, multifetal pregnancy and invasive procedures like cerclage or amniocentesis [2]. Although the underlying pathophysiology remains largely undefined, subclinical infection has been implicated in PPRM. Microorganisms originating in the vagina or cervix may colonize tissues such as decidua and possibly fetal membranes. Lipopolysaccharide elaborated by these bacteria

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induces proinflammatory cytokine production (TNF- α and IL-1) by amniotic epithelial cells, chorionic cells and tissue macrophages. Inflammation–oxidative stress axis plays a major role in membrane weakening through a variety of processes. In addition, proteases released by some colonizing microbes have a direct effect on the fetal membranes [3].

Bacterial vaginosis (BV) as well as aerobic vaginitis (AV) are defined as disequilibrium in the vaginal microflora. Bacterial vaginosis can be asymptomatic or diagnosed on examination for other indications [4].

Aerobic vaginitis is characterized by abnormal vaginal microflora accompanied by an increased localized inflammatory reaction. The aerobic vaginitis during pregnancy gives rise to perinatal complications, such as preterm birth (PTB), PROM, and fetal infection [5].

Failure of proper diagnosis of AV or erroneous diagnosis as BV may lead to complications of aerobic vaginitis such as desquamative inflammatory vaginitis, which increases the risk of preterm delivery, chorioamnionitis during pregnancy [6].

As diagnosis and treatment of vaginal infections in our resource constrained setting depend mainly on clinical signs and symptoms of the patients not as a part of routine antenatal care, this study aimed to assess the relation of vaginal infections and its antimicrobial profile with PPRM and pregnancy outcome.

Materials and Methods

Study design and participants

This case control study was carried out at an antenatal clinic of a secondary care hospital (Zagazig General Hospital), emergency hospital of obstetrics and gynecology in Zagazig University Hospitals (tertiary care hospital) and microbiological evaluation was done at Medical Microbiology and Immunology Department of Zagazig University during the period from February 2019 to February 2020.

Population of the study

Pregnant women with single live fetus at period of gestation 28- 37 weeks as calculated by last menstrual period or by ultrasound, with either intact membrane “control group- n= 320” or PPRM “case group- n = 320”

Case definition of PPRM included history of sudden gush of fluid with continued leakage. Evidence of fluid pooling in the vagina or leaking from the cervical os when the patient coughs or when fundal pressure is applied on vaginal examination. In addition to ultrasonography parameters [7].

Exclusion criteria

Pregnant female with known obstetrical conditions, which can be a confounding factor for premature rupture of membrane or preterm labor such as antepartum hemorrhage, multiple gestation, polyhydramnios, structural and functional abnormalities of the uterus. Pregnant females with history of Infections (diarrhea or urinary tract infections), sexual intercourse within last 24 hours, vaginal bleeding and antibiotic treatment within the previous 4 weeks were also excluded. Participants with *Trichomonas vaginalis* (TV) infection as well as patients with unreadable or unclassifiable examined vaginal swab were excluded.

Ethical approvals

The study complied with the guidelines of the Declaration of Helsinki 1975. Written informed consent was obtained from all participants at the start of the study. The study was approved by the Zagazig University Institution Review Board (ZU-IRB) (Approval code 5667).

Work up

1) Clinical evaluation

Detailed history was obtained by interviewing the participant with special concern to current pregnancy (history of vaginal discharge, antepartum bleeding), past history (history of previous preterm labor, PROM, recurrent vaginal infection), medical history (diabetes, chest diseases) and menstrual history (to calculate gestational age). General examination including (pulse, blood pressure, temperature), abdominal examination (fundal level, fetal heart sounds and uterine contractions) and ultrasound examination (assess fetal viability, gestational age, fetal weight, amount of liquor and placental site).

2) Vaginal examination, vaginal pH assessment and Whiff test

This was done by introducing sterile unlubricated Cusco’s vaginal speculum under complete aseptic conditions. Nature (color, consistency, and odor) of the discharge, condition of the vagina wall “redness or ulceration” and cervix were noted. Vaginal pH was assessed by nitrazine pH paper.

Bacterial vaginosis was identified clinically for control group by Amsel criteria by presence of the following criteria upon vaginal examination (Increased homogeneous thin vaginal discharge; pH of the secretion greater than 4.5; Amine odor when potassium hydroxide 10% solution was applied to vaginal secretions). Vaginosis evaluation was completed upon microscopic examination of the swab in the laboratory. Aerobic vaginitis was suspected clinically by vaginal redness, oedema or erosions and confirmed by aerobic vaginitis score.

Clinical diagnosis of bacterial vaginosis in case group was infeasible due to amniotic fluid interference with both visualization of discharge and pH assessment. Diagnosis was confirmed by microscopic examination of vaginal swab.

3) Microbiological work up

Sample collection processing and transport

Three high lateral vaginal swabs were obtained to collect vaginal discharge from each participant. Few drops of 10% KOH were applied to the first swab to detect fishy odor (Whiff test) in the clinic. The other two swabs were transported within an hour to the microbiology laboratory for further microbiological assessment.

Microscopic examination and interpretation

A swab was spread in onto two slides and mixed with a drop of saline on one slide to exclude trichomonas infection and detect clue cells. The second slide was Gram-stained for Nugent scoring and detection of candida.

Nugent's scoring was done by examination of a minimum of 10 high power fields (1000x) for three bacterial morphotypes including *Lactobacillus spp.* (large Gram-positive rods), *Gardnerella/Bacteroides* (small Gram-negative rods) and *Mobiluncus spp.* (curved Gram-variable rods). These three morphotypes were quantified on a scale of 1-4. Individual morphotype was scored and the total score was obtained by the addition of these three different scores. Generally, Nugent score of 0–3 is consistent with a *Lactobacillus*-predominant vaginal microbiota, 4–6 with intermediate microbiota (emergence of *G. vaginalis*), and 7–10 with BV [8]. An intermediate microbiota remains an uncharacterized category and is a challenge in the diagnosis of BV due to unknown clinical implications and according to **Thomason and coworkers** [9] who stated that clue cells on wet mount examination of vaginal secretions is the single most reliable indicator of

bacterial vaginosis. Bacterial vaginosis was diagnosed if Nugent's grading scored ≥ 7 or 4-6 in the presence of Clue Cells. Scores from 0 to 3 and 4-6 (without Clue Cells) were considered inconsistent with bacterial vaginosis [10].

Aerobic vaginitis score was determined by saline wet mount microscopic examination of vaginal swab for lactobacilli grade, background flora, number of leukocytes, proportion of toxic lymphocyte and parabasal epitheliocytes. Aerobic vaginitis score of < 3 indicates absence of AV [11].

Vaginal candidiasis was diagnosed by Gram's staining for the presence of budding yeast cells and pseudo-hyphae of *Candida* species.

Vaginal swab culture, pathogen identification and antimicrobial susceptibility

The last vaginal swab was inoculated on MacConkey, blood agar, chocolate agar and Sabouraud dextrose agar (SDA). MacConkey, blood agar and SDA plates were incubated aerobically. Chocolate agar plates were incubated in candle jar for 24–48 h at 35–37°C. Plates were checked for significant growth either pure or mixed (Heavy growth of vaginal potential pathogen was defined as growth in the third or fourth zone on the agar plate) [12]. Identification of isolates was done by conventional methods, catalase negative Gram-positive cocci were further identified by latex agglutination test using PASTOREXTM STREP (Bio-Rad, Marnes-la-Coquette, France). Yeast growth was identified by Germ tube formation test for identification of *Candida albicans* [13].

Antibiotic susceptibility testing was performed for isolated bacteria using the disk diffusion method according to CLSI guidelines. For Gram positive bacteria, the tested antibiotics included penicillin G (10 units), ampicillin (10 µg), ceftriaxone (10 µg), erythromycin (15 µg), clindamycin (2 µg) and vancomycin (30 µg). For Gram negative bacteria, the tested antibiotics included Ampicillin (10 µg), amoxicillin-clavulanic acid (20 / 10 µg), ceftriaxone (30 µg), Cefuroxime (30 µg), Gentamicin (10 µg), and imipenem (10 µg) by disk diffusion method. *P. aeruginosa* ATCC®27853, *Staphylococcus aureus* ATCC®25923 were used as a quality control strains (American Type Culture Collection [ATCC], Manassas, VA, USA).

Culture and susceptibility of anaerobic bacteria were not done as most are fastidious need special culture media, difficult to identify by conventional methods

and metronidazole is an effective drug for most anaerobes [14].

Participant management and maternal outcome assessment

All participants diagnosed with PPRM were admitted to hospital with continuous monitoring for labor and fetal distress. Oral erythromycin 250 mg administration, 4 times daily for 10 days, unless labor was established. In case of erythromycin unavailability amoxicillin was prescribed. Pregnant females with PPRM at <35 weeks of gestation were administered intramuscular dexamethasone to enhance fetal lung maturity, in accordance with the NICE guideline adopted by the Royal College of Obstetricians and Gynecologists [15]. Oral metronidazole course was prescribed for participants with BV. In case of significant bacterial growth, antibiotic was prescribed according to culture and sensitivity results.

Admitted patients were serially evaluated clinically and by laboratory results for amnionitis and fetal well-being. Patients were managed according to the findings. Maternal and fetal outcome were followed up and recorded for a month after delivery.

Statistical analysis

After data collection, data were coded, entered, and analyzed using SPSS (Statistical Package for Social Science) version 25. Qualitative data were presented as frequencies and percentages while, quantitative data were presented as mean, standard deviations (SD). Qualitative independent data were compared using Chi square test. Also, we used odds ratio (OR) for risk assessment in case control study in which we measure association between exposure and outcome. p value (≤ 0.05) was considered statistically significant difference and <0.001 is considered high statistically significant difference.

Results

A total of 640 pregnant participants were enrolled (320 PPRM cases and 320 controls). No statistically significant difference between both studied groups regarding maternal age, gestational age, and parity. Diabetes mellitus was more prevalent in case group with statistical significance ($p < 0.001$) (Table 1).

Isolated BV and AV were identified in 29.1% and 17.3% of all participants respectively. Prevalence of BV was 42.2% in case group and 10.3% in control group with statistically significant difference. Concerning AV prevalence among

PPROM patients is 33.4% and only 1.2% among females with normal pregnancy with statistical significance ($p < 0.001$). Aerobic vaginitis and vaginal candidiasis were major risk factors for PPRM (odds ratio = 39.7, 13.5 respectively). Isolated Vaginal candidiasis was detected in 7.8% of case group and 0.6% of control group, all of them were diabetic females. No mixed vaginal infection was detected in control group and only 3.4% of cases had mixed vaginal infections. Normal vaginal flora was observed microscopically in only 20.6% of cases and 80.3% of controls with significantly less normal flora among PPRM pregnant females ($p < 0.001$) (Table 2).

Among PPRM cases with confirmed BV, only 31/111 (27.9%) gave history of symptoms consistent with vaginosis before rupture of membrane, similarly only 10/57 (17.5%) of control group with vaginosis were symptomatic. Most participants with aerobic vaginosis, 96.7% and 100% of PPRM and controls were symptomatic.

Evaluation of vaginal swab culture showed that no significant aerobic bacterial growth in 15.6% of cases and 75% of control group participants ($p < 0.001$). *Streptococcus agalactiae* (*S. agalactiae*) was the most prevalent pathogen isolated from 11.6% of pregnant females with intact fetal membranes and was the most prevalent overall organism isolated from 120 (18.8%) of all participants. On the other hand, *E. coli* and *S. agalactiae* were nearly equally isolated from 25.9% and 25% of case group (Table 3). The overall Gram-positive bacteria was isolated in 33.4% of participants while Gram-negative bacteria was isolated from 21.7% of them.

Streptococcus agalactiae Isolates were susceptible to all tested antimicrobials except 11.7% that were resistant to erythromycin. Linezolid was more effective than vancomycin against enterococci while erythromycin was the least effective against Gram positive isolates where 50%, 25% and 16.7% of *Enterococci*, *S. aureus* and *S. agalactiae* respectively were resistant to it. *E. coli* and *K. pneumoniae* isolates were most susceptible to ceftriaxone 89/115 (77.4%) and 16/22 (72.2%) respectively. Ampicillin was the least effective antibiotic against Gram negative bacterial isolates with susceptibility rate of only 43.5% of *E. coli* and 45.5% *K. pneumoniae* (Table 4).

Febrile morbidity during pregnancy and post partum purperal sepsis was most prevalent in aerobic vaginitis cases reported in 56.1% of cases followed by mixed vaginitis and candidiasis. Only

14.3% of PPRM pregnant females with normal flora developed fever during pregnancy, non of them progressed to purperal sepsis (Table 5).

Table 1. Basic characteristics of studied groups.

Variables		Cases (No.=320) Mean±SD		Controls (No.=320) Mean±SD		T test	p value
Age (years) Mean± SD		28.7 ± 9.03		29.9 ± 10.8		1.524	0.254
Gestational age (weeks) Mean± SD		32.9 ± 12.21		31.97 ± 12.8		0.941	0.694
		No.	%	No.	%	X2	p value
Parity, n (%)	PG	141	44.1	153	47.8	0.906	0.341
	Multipara	179	55.9	167	52.2		
Diabetes mellitus		43	13.4	9	2.8	24.196	<0.001**

T test=Independent t-test. # X2= Chi square test

**=Highly significant

Table 2. Prevalence of vaginal infections and normal vaginal flora among case and control groups assessed microscopically.

	Cases (n=320)		Controls (n=320)		X2 Test	OR (95% CI)	p. value
	No.	%	No.	%			
BV, n (%) (No.=186, 29.1%)	135	42.2	33	10.3	8397	6.3(4.2-9.6)	<0.001**
AV, n (%) (No.=111, 17.3%)	107	33.4	4	1.2	115.6	39.7(14.4-109.3)	<0.001**
vaginal candidiasis (No.=27, 4.2%)	25	7.8	2	0.6	20.45	13.5(3.2-57.4)	<0.001**
Mixed vaginitis (No.=11, 1.7%)	11	3.4	0	0.0	11.19	-----	<0.001**
Normal flora (No.=323, 50.1%)	66	20.6	257	80.3	357.03	0.02(0.01-0.03)	<0.001**

Bacterial vaginosis (BV), Aerobic vaginitis (AV)

Table 3. Revealed significant microbial growth in culture of vaginal swab in case and control groups.

Type of microorganism	Case (No.=320)		Control (No.=320)		X ²	p value
	No.	%	No.	%		
<i>S. agalactiae</i> (No.=120)	83	25.9	37	11.6	21.702	<0.001**
<i>E. coli</i> (No.=115)	80	25.0	35	10.9	21.46	<0.001**
<i>Staphylococcus aureus</i> (No.=54)	54	16.9	0	0.0	58.97	<0.001**
<i>Enterococci & group D streptococci</i> (No.=40)	40	12.5	0	0.0	42.66	<0.001**
<i>Klebsiella pneumoniae</i> (No.=24)	24	7.5	0	0.0	24.93	<0.001**
<i>Candida species</i> (No.=38)	36	11.3	2	0.6	32.34	<0.001**
No aerobic pathogen (No.=290)	50	15.6	240	75.0	227.6	<0.001**
Gram positive bacteria (No.=214)	177	55.3	37	11.6	137.59	<0.001**
Gram negative bacteria (No.=139)	104	32.5	35	10.9	43.75	<0.001**

X²=Chi square test. **=Highly significant**Table 4.** Antimicrobial susceptibility of isolated bacteria.

	<i>S. agalactiae</i> (No.=120)		<i>E. coli</i> (No.= 115)		<i>S. aureus</i> (No.=50)		<i>Enterococci</i> (No.=40)		<i>K. pneumoniae</i> (No.=22)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Penicillin	120	100.0	NA	-----	10	20.0	14	35.0	NA	-----
Ampicillin	120 [#]	100.0 [#]	50	43.5	10 [#]	20.0 [#]	16	40.0	10	45.5
Amoxicillin-clavulanic acid	120 [#]	100.0 [#]	76	66.1	10 [#]	20.0 [#]	21	52.5	12	54.5
Ceftriaxone	120 [#]	100.0 [#]	89	77.4	10 [#]	20.0 [#]	14	35.0	16	72.7
Vancomycin	120	100.0	NA	-----	50	100.0	37	92.5	NA	-----
Linezolid	120	100.0	NA	-----	50	100.0	40	100.0	NA	-----
Erythromycin	106	88.3	NA	-----	43	86.0	20	50.0	NA	-----
Clindamycin	120	100.0	NA	-----	50	100.0	40	100.0	NA	-----

NA (not applicable: there is no suggested zone diameters in CLSI guidelines)

[#] According to CLSI guidelines all penicillin susceptible isolates are considered susceptible for other beta lactams**Table 5.** Maternal and neonatal infectious complications in PPRM cases.

	BV, n (%) (No.=101)		AV, n (%) (No.=107)		Vaginal candidiasis (No.=25)		Mixed vaginitis (No.=11)		Normal flora (No.=42)		X ²	p.value
	No.	%	No.	%	No.	%	No.	%	No.	%		
Febrile morbidity during pregnancy (No.=99)	20	19.8	60	56.1	9	36.0	4	36.4	6	14.3	53.97	<0.001**
Puerperal sepsis (No.=75)	12	11.9	56	52.3	7	28.0	0	0.0	0	0.0	41.418	<0.001**
Early onset neonatal infections (No.=76)	16	15.8	54	50.5	4	16.0	1	9.1	1	2.4	65.903	<0.001**

X²=Chi square test. **=Highly significant

Discussion

Spontaneous PPROM accounts for approximately 70 to 80% of all preterm births. Preterm birth is strongly associated with significant neonatal morbidity, mortality, and long-term disability [7]. Therefore, the ability to predict and manage the risk factors of spontaneous preterm delivery including vaginal infection has important clinical implications for a better neonatal, and maternal outcome [16]. Although management of both mother and newborn in PPROM is directed by international guidelines, PROM prevention and management still appear to be controversial both as regards the definition of the risk factors and the validity of a preventive treatment.

There was no significant difference in occurrence of PPROM among different maternal age groups, parity, and duration of pregnancy. Like **Brown and coworkers** [3] who studied the risk of vaginal flora imbalance on preterm fetal membrane rupture and found that there was no significant difference in maternal age regarding both vaginal flora imbalance and membrane rupture. In contrast to **Darine and coworkers** [17] who reported maternal advanced age as a risk factor of PPROM. Higher prevalence of diabetes mellitus in PPROM group than control group was in line with other studies [18].

Many factors can influence the vaginal microbial composition, such as nutrition, hormonal fluctuations during pregnancy, sexual and hygienic practices [19]. Lactobacilli flora was visualized in Gram-stained vaginal swab in only 13.1% of PPROM cases and diminished or completely absent in 86.9 % of them with predominance of other organisms including that of bacterial vaginosis, aerobic vaginitis, and vaginal candidiasis. Absence or marked reduction of lactobacilli increases the incidence of PPROM [3].

Vaginal microbial changes are associated with an increase in local activation of the innate immune system and induction of the inflammatory cascade, which may induce membrane disruption with preterm labor or PPROM [20]. In current study normal flora was significantly prevalent in pregnant female with normal pregnancy. Small percentage of PPROM cases had normal vaginal flora at time of presentation indicating that non-infectious mechanism is likely responsible for rupture of membrane in these cases.

Bacterial vaginosis in our study was reported in overall 29.1% with 10.3 % prevalence in normal pregnancy. Vaginosis prevalence among Egyptian pregnant females reported in different studies was variable 33.3% and 25.26% [21,22]. **Darwish et al.** [21] selectively screened high risk women, with a history of prior idiopathic preterm labor or second trimester miscarriage, while **Gohar and Shazly** [22] examined female in the first trimester to detect relation between vaginosis and abortion.

Bacterial vaginosis in current study was a risk factor for PROM (OR=6.3). Some studies showed that BV was not associated with PROM [17,23], although it is the commonest vaginitis in term pregnancy. The US Preventive Services Task Force (USPSTF) in their 2020 update reported moderate certainty that asymptomatic bacterial vaginosis in pregnant female not a risk for preterm delivery and that screening asymptomatic pregnant females has no net benefit in preventing preterm delivery [24].

On the other hand, other studies stated that BV increased the risk of developing PPROM [25]. The difference in the results of different studies regarding the increased risk of adverse reproductive and pregnancy outcomes including PPROM might be owed to BV-related bacterial species rather than BV itself. Furthermore, it has been noted that mothers who are truly at risk of PPROM and/or preterm labor need both an environmental factor and a genetic predisposition to an inflammatory response that leads to infection associated PPROM [26].

Aerobic vaginitis is characterized by disruption of normal *Lactobacilli* flora and overgrowth of aerobic microorganisms with increased inflammatory response in vaginal epithelium [27].

Aerobic vaginitis was a significant risk factor of PPROM (OR= 39.7). This is in line with other studies [1,27,28]. The marked inflammatory response in aerobic vaginitis diminishes membrane tension, leading to rupture of membrane. This may elucidate the increased prevalence of PPROM among females with aerobic vaginitis than those with bacterial vaginosis.

In our study vaginal candidiasis was higher among PPROM cases in contrast to females with normal pregnancy (OR= 13.5) in contrast to the study done by **Darine and coworkers** [17] there was no association between the vaginal candidiasis

or any other genital infection and premature rupture of membranes among pregnant Tunisian women [17].

Mixed vaginitis in accounted for only 1.7% of vaginal infection in current study. Higher prevalence of mixed vaginal infection was reported in other studies [29].

Heavy bacterial growth from vaginal swab in our study showed highest prevalence of *S. agalactiae* (18.8% of participants). Vaginal carrier rate of *S. agalactiae* among Egyptian pregnant females was reported in a previous study to be 23.5% [30]. In our study it was revealed in vaginal swab of 25% of pregnant females with PPROM. Much higher prevalence of GBS was reported by **Gebriel et al.** [31] who isolated it from 66 % of PPROM cases.

Escherichia coli. Prevalence in current study is slightly lower than prevalence of GBS in case and control groups. Different studies reported different bacterial prevalence in vaginal swab of PPROM Cases. **Elsherief and Ahmed** [32] reported *E. coli* significantly more prevalent in PPROM cases, followed by group B Streptococci (GBS). Bacterial prevalence in vaginal infection differs among the different geographical regions. This variation in isolates leads to lack of standardized treatment for infected mothers to reduce the rate of adverse pregnancy outcomes.

In-vitro antimicrobial susceptibility of GBS isolates revealed erythromycin resistance in 11.7% versus susceptibility to all other tested antimicrobials including ampicillin and amoxicillin-clavulanic acid. Erythromycin is the recommended drug for management of PPROM cases based on results from ORACLE I trial, as it caused prolongation of pregnancy for 48 hours and reduce neonatal morbidity in women randomized to orally administered erythromycin prophylaxis compared to placebo [33]. This may be attributable to its antibacterial activity at the placental tissues or its anti-inflammatory effect and tocolytic action proved by some studies *in-vitro* [3].

In current study, amoxicillin-clavulanic acid showed reasonable *in-vitro* effect on *E. coli*, *Enterococci*, and *K. pneumoniae* while erythromycin lack antimicrobial action against Gram-negative bacteria and does not provide adequate control of GBS. **Brown and coworkers** [3] in their study stated that in women who experience PPROM, erythromycin therapy caused shift of vaginal microbiota to dysbiosis, lactobacilli

depletion and increased prevalence of chorioamnionitis and neonatal infections.

Gram-negative bacteria were isolated from vaginal swabs in 32.5% of PPROM cases in our study, such percentage of potential pathogen isolates should not be neglected during PPROM management. **Gebriel and coworkers** [31] in their *in-vivo* evaluation of erythromycin versus amoxicillin use in PPROM management reported better fetal and maternal outcome in amoxicillin treated group. **Sobel and coworkers** [34] reported clindamycin to be a better choice for treating pregnant women with disturbed vaginal microbiota due to its ability to eradicate both AV and BV-associated bacteria.

The benefit of screening and treatment for abnormal vaginal microbiota to reduce PPROM remain debatable. Although Interventions to prevent pre-term birth or PPROM shows ineffectiveness probably due to the increasing antimicrobial resistance as well as the misdiagnosis of AV as BV, thus leading to treatment failures [35].

Concerning maternal and neonatal infection, the highest prevalence of such complications was reported in AV group. PPROM with vaginosis showed lower maternal febrile complications. Other studies reported increased prevalence of maternal and neonatal complications in PPROM cases with aerobic vaginosis [27,36].

Conclusion

Vaginal microbiome imbalance and colonization by potential pathogens carry risk of PPROM and adverse maternal and neonatal outcomes. Accurate diagnosis of vaginal infection, precise medical treatment during pregnancy is essential for maintenance of maternal and neonatal health.

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