Pathogens in preterm prelabour rupture of membranes and erythromycin for antibiotic prophylaxis: a retrospective analysis

YY Li, CW Kong *, William WK To

A B S T R A C T

Introduction: Many authoritative guidelines recommend prescribing erythromycin as antibiotic prophylaxis in patients with preterm prelabour rupture of membranes (PPROM). This study evaluated the spectrum of pathogens in PPROM and assessed the effectiveness of erythromycin prophylaxis.

Methods: This retrospective study enrolled pregnant patients who were diagnosed with PPROM and who delivered at ≥24 weeks of gestation in an obstetric unit from 2013 to 2017. Pathogens isolated from maternal, placental, and neonatal specimens were analysed; their sensitivity profiles to various antibiotics were recorded. Neonatal outcomes were also evaluated.

Results: The overall incidence of PPROM was 2.63%. Gram-positive bacteria were cultured in 18.4% of PPROM patients (most frequent: Group B *Streptococcus* [GBS; 14.6%]); Gram-negative bacteria were cultured in 12.8% of PPROM patients (most frequent: *Escherichia coli* [8.0%]). Both Gram-positive and Gram-negative bacteria were significantly associated with early-onset neonatal sepsis (P=0.036 and P=0.001). In analyses stratified by bacterial species, *E coli* was significantly associated with early-

onset neonatal sepsis (P=0.004), whereas GBS was not (P=0.39). Gram-positive bacteria had high rates of resistance to common antibiotics: 42.2% of GBS and 50.0% of *Enterococcus* and other *Streptococcus* bacteria were resistant to erythromycin. *Escherichia coli* had high rates of resistance to ampicillin (70.3%) and gentamicin (33.3%); rates of resistance to co-amoxiclav (3.6%) and intravenous cefuroxime (14.0%) were low.

Conclusion: Gram-positive and Gram-negative bacteria were found in 29.1% of PPROM patients. Administration of erythromycin alone was insufficient to control these bacteria in 67.7% of patients with positive cultures.

YY Li, MB, ChB
CW Kong *, MB, ChB, MSc
WWK To, MD

Department of Obstetrics and Gynaecology, United Christian Hospital, Kwun Tong, Hong Kong

* Corresponding author: melizakong@gmail.com

New knowledge added by this study

- Gram-positive and Gram-negative bacteria were found in 29.1% of patients with preterm prelabour rupture of membranes (PPROM), and the presences of these bacteria were significantly associated with the development of early-onset neonatal sepsis.
- Erythromycin alone is insufficient to control the growth of Gram-positive and Gram-negative bacteria in patients with PPROM. In particular, *Escherichia coli* and Group B *Streptococcus* isolates showed high rates of resistance to erythromycin.

Implications for clinical practice or policy

- Based on the increase in Gram-negative bacteria and the association of these bacteria with early-onset neonatal sepsis, intravenous cefuroxime (a second-generation cephalosporin) is proposed for use as antibiotic prophylaxis, in combination with erythromycin, in patients with PPROM.

Introduction

Preterm prelabour rupture of membranes (PPROM) occurs in 2.0% to 3.5% of pregnancies and contributes to 30% to 40% of all preterm births.[1] Importantly, PPROM is directly associated with preterm labour, prematurity, chorioamnionitis, maternal and neonatal infections, and adverse maternal and neonatal outcomes.[2] Patients with

PPROM reportedly have a higher rate of abnormal microbial colonisation of the genital tracts than patients without PPROM; the prevalence of positive amniotic-fluid cultures in PPROM patients is approximately 32% to 35%.[1] Administration of antibiotics in PPROM patients has been shown to significantly reduce clinical chorioamnionitis; delay the onset of delivery; decrease neonatal infection;

未足月胎膜早破的病原體和以紅黴素作抗生素預防療法：回顧性分析

李恩瑜、江采華、杜榮基

引言：很多權威指引都推薦使用紅黴素作為未足月胎膜早破（PPROM）病人的抗生素預防療法。本研究評估PPROM的病原體系列和探討紅黴素的預防功效。

方法：這項回顧性研究納入2013年至2017年在一個產科部門出現PPROM並且在懷孕24週後生產的病人。我們分析從母體、胎盤及初生嬰兒樣本中培植出來的病原體，並且記錄它們對多種抗生素的敏感性。初生嬰兒的產後狀況也一併分析。

結果：PPROM的總體發生率是2.63%。有18.4% PPROM病人培植出革蘭氏陽性菌（當中最常見的是B型鏈球菌，佔14.6%）；12.8% PPROM病人培植出革蘭氏陰性菌（當中最常見的是大腸桿菌，佔8.0%）。革蘭氏陽性菌和陰性菌都與早發性新生兒敗血症有重要關係（P=0.036，P=0.001）。當每種病原體獨立分析，我們發現大腸桿菌與早發性新生兒敗血症有重要關係（P=0.004），但B型鏈球菌則沒有（P=0.39）。革蘭氏陽性菌對常用的抗生素呈抗藥性：42.2%的B型鏈球菌、50%的腸球菌及其他鏈球菌都對紅黴素呈抗藥性。大腸桿菌對氨苄青黴素及慶大霉素呈很高抗藥性（分別佔70.3%及33.3%），但對阿莫西林克拉維酸鉀及靜脈注射頭孢呋辛則呈低抗藥性（分別佔3.6%及14.0%）。

結論：我們在29.1% PPROM病人中發現有革蘭氏陽性菌和陰性菌。紅黴素沒法壓制當中67.7%培植出的細菌。

and reduce the use of surfactant, oxygen therapy, and abnormal neonatal cerebral ultrasound prior to discharge from hospital.[3]

A randomised controlled trial published in 1997 showed that the use of erythromycin and ampicillin as antibiotic prophylaxis in PPROM patients could significantly reduce neonatal morbidity.[4] In 2001, the landmark randomised controlled trial ORACLE 1 showed that the use of erythromycin could significantly prolong pregnancy in PPROM patients and could improve neonatal outcomes.[1] Based on the above two trials, many authoritative guidelines recommend prescribing erythromycin with or without ampicillin for PPROM patients, including guidelines from the Royal College of Obstetricians and Gynecologists,[5] the American College of Obstetricians and Gynecologists,[6] the Society of Obstetricians and Gynaecologists of Canada,[7] and the Royal Australian and New Zealand College of Obstetricians and Gynaecologists.[8]

However, more recent studies have found that colonisation by Gram-negative bacteria, especially *Escherichia coli*, has been increasing in PPROM patients, such that these bacteria constitute a significant proportion of all pathogens involved in cases of PPROM and early-onset neonatal sepsis.[9,10] Because the above two trials were conducted nearly 20 years ago, the objective of this study was to re-evaluate the pathogens involved in PPROM and characterise their respective sensitivity profiles to guide the appropriate choice of antibiotics used for optimal control, and to assess whether erythromycin remains an effective antibiotic to control these pathogens in PPROM patients.

Methods

This was a retrospective analysis of a cohort of all pregnant patients who were diagnosed with PPROM and who delivered at ≥24 weeks of gestation in United Christian Hospital from 1 January 2013 to 31 December 2017. These patients were identified and retrieved from the labour ward registry. Diagnoses of PPROM were made based on clinical history and speculum examination to determine the presence of liquid leaking from the cervical os; ultrasound was performed when necessary to aid the diagnosis of PPROM. In accordance with our department protocol, all patients who were diagnosed with PPROM underwent microbiological investigation, including high vaginal swab and mid-stream urine for bacterial culture, and low vaginal swab and rectal swab for Group B *Streptococcus* (GBS) culture. Maternal blood culture was performed if maternal fever or signs of acute chorioamnionitis were observed. Microbiological investigation was repeated when clinically indicated. All patients who were diagnosed with PPROM were administered oral erythromycin 250 mg, 4 times per day for 10 days, unless labour was established; patients 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 Gynaecologists.[5] Conservative management was adopted for patients at <34 weeks of gestation, unless there was evidence of acute chorioamnionitis or preterm labour was established. Possible induction of labour was discussed with patients at ≥34 weeks of gestation. Caesarean section was performed in accordance with obstetric indications. Erythromycin was changed to another appropriate antibiotic if culture results demonstrated the presence of erythromycin-resistant bacteria. If a patient had spontaneous or induced labour, intravenous benzyl penicillin was administered to control GBS until the baby was delivered. For all PPROM patients, placental swabs were sent for bacterial culture and the placentae were sent for histology examination after delivery. All neonates were assessed by paediatricians after birth and appropriate neonatal cultures were taken as indicated. Regardless of the presence of positive bacterial cultures, neonates were diagnosed with early-onset neonatal sepsis if they had signs of systemic infection within 72 hours after birth; these signs included unstable body temperature, lethargy or irritability, feeding intolerance, respiratory

distress, tachycardia or hypotension, metabolic changes (eg, glucose level and acidosis), neutropenia, or increased acute-phase reactants (eg, C-reactive protein).

The demographic and clinical data of the pregnant patients and their neonates were retrieved from a comprehensive obstetric database and the Clinical Management System of the Hospital Authority. The SPSS (Windows version 20.0; IBM Corp, Armonk [NY], United States) was used for data entry and analysis. Continuous variables were analysed by t test, whereas discrete variables were analysed by the Chi squared test or Fisher's exact test. A P value of <0.05 was considered to be statistically significant. The STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines were followed in the preparation of this article.[11]

Results

In total, there were 21 047 pregnancies with 21 375 babies delivered during the study period, including 324 pairs of twins and two sets of triplets. The incidence of PPROM was 2.63% (553/21 047), including 39 pairs of twins. The demographic data and pregnancy outcomes of patients with and without PPROM are shown in Table 1. Advanced maternal age, primiparity, and multiple pregnancies were more common among patients in the PPROM group than among patients in the non-PPROM group. Patients with PPROM delivered earlier (34.1 vs 38.8 weeks; P<0.0001) and had lower birthweight babies (2260 g vs 3142 g; P<0.001) than patients without PPROM. The incidence of neonatal death was higher in the PPROM group than in the non-PPROM group (1.4% vs 0.1%; P<0.001), whereas the incidence of stillbirth did not significantly differ between the two groups (0.3% vs 0.3%; P=0.73).

The outcomes of patients with PPROM and the types of bacteria cultured from maternal, placental, and neonatal specimens are shown in Table 2. Gram-positive bacteria were found in 18.4% of PPROM patients, among which GBS was the most common (14.6%); Gram-negative bacteria were found in 12.8% of PPROM patients, among which E $coli$ (8.0%) was the most common. In addition, anaerobes were found in 1.8% of PPROM patients. Although 19.2% of placental histology specimens showed evidence of chorioamnionitis or funisitis, only 4.7% of PPROM patients were clinically diagnosed with acute chorioamnionitis. However, early-onset neonatal sepsis was diagnosed in 10.8% of newborns.

Preterm prelabour rupture of membranes occurred earlier in gestation in patients with early-onset neonatal sepsis than in those without neonatal sepsis (31.1 vs 34.2 weeks; P<0.001), and the PPROM to delivery interval was longer in patients with early-onset neonatal sepsis (3.7 vs 1.5 days; P<0.001). The presences of Gram-positive bacteria and Gram-negative bacteria were significantly associated with the development of early-onset neonatal sepsis (P=0.036 and P=0.001, respectively), whereas the presence of anaerobes was not (P=0.08). In addition, the presence of E $coli$ was significantly associated with the development of early-onset neonatal sepsis (P=0.004), whereas the presence of GBS was not (P=0.39) [Table 3].

The rates of resistance of Gram-positive bacteria and Gram-negative bacteria to various antibiotics are shown in Tables 4 and 5, respectively. All GBS isolates were sensitive to penicillin, but 42.2% of them were resistant to erythromycin; other $Streptococcus$ and $Enterococcus$ isolates also showed resistance to erythromycin (50% of each group). $Escherichia$ $coli$ isolates demonstrated high rates of resistance to ampicillin (70.3%) and gentamicin

TABLE 1. Demographic data and pregnancy outcomes of patients with and without PPROM*

Demographic data	Patients with PPROM (n = 553)	Patients without PPROM (n = 20 494)	P value (95% CI)
Maternal age	33.0 ± 5.3	32.1 ± 5.2	<0.001 (0.39-1.26)
Advanced maternal age (≥35 years)	219 (39.6%)	6712 (32.8%)	0.001
Primiparous	332 (60.0%)	9958 (48.6%)	<0.001
Multiple pregnancy	39 (7.1%)	287 (1.4%)	<0.001
Caesarean delivery	142 (25.7%)	4687 (22.9%)	0.12
Gestation at delivery (weeks)	34.1 ± 2.6	38.8 ± 1.75	<0.001 (-4.83 to -4.53)
Birthweight (g)†	2260 ± 547	3142 ± 481	<0.001 (-921.37 to -842.49)
Stillbirth†	2 (0.3%)	69 (0.3%)	0.73
Neonatal death†	8 (1.4%)	20 (0.1%)	<0.001

Abbreviations: CI = confidence interval; PPROM = preterm prelabour rupture of membranes
* Data are shown as mean ± standard deviation or No. (%), unless otherwise specified
† There were 592 babies in the group with PPROM and 20 783 babies in the group without PPROM

TABLE 2. Pregnancy outcomes and microbiology investigation results in women with PPROM (n = 553)*

	Value†
Pregnancy outcomes	
Gestation at PPROM (weeks)	33.5 ± 2.9
Gestation at delivery (weeks)	34.1 ± 2.6
PPROM to delivery interval (days)	1.7 ± 4.5
Clinical diagnosis of acute chorioamnionitis	26 (4.7%)
Chorioamnionitis with and without funisitis from pathological examination	106 (19.2%)
Early-onset neonatal sepsis	60 (10.8%)
Microbiology investigation results	
Gram-positive bacteria found in maternal, placental, or neonatal cultures	102 (18.4%)
Group B *Streptococcus*	81 (14.6%)
Other *Streptococcus*	9 (1.6%)
Enterococcus	6 (1.1%)
Staphylococcus	6 (1.1%)
Gram-negative bacteria found in maternal, placental, or neonatal cultures	71 (12.8%)
Escherichia coli	44 (8.0%)
Klebsiella	9 (1.6%)
Bacteroides	5 (0.9%)
Proteus	4 (0.7%)
Prevotella	4 (0.7%)
Citrobacter	2 (0.4%)
Enterobacter	1 (0.2%)
Morganella	1 (0.2%)
Aggregatibacter aphrophilus	1 (0.2%)
Anaerobes found in maternal, placental, or neonatal cultures	10 (1.8%)
Peptostreptococcus	7 (1.3%)
Bifidobacterium dentium	1 (0.2%)
Peptoniphilus harei	1 (0.2%)
Clostridium	1 (0.2%)

Abbreviation: PPROM = preterm prelabour rupture of membranes
* Data are shown as mean ± standard deviation or No. (%) of patients
† Fourteen patients had mixed bacterial cultures, of which 12 had mixed Gram-positive and Gram-negative bacterial cultures. Positive bacterial culture cases are listed

literature.[1] The identified risk factors for PPROM included advanced maternal age, primiparity, and multiple pregnancies. Our cohort showed that women with PPROM had greater incidences of preterm deliveries, lower birthweight babies, and neonatal death, confirming a relationship between PPROM and major neonatal morbidity and mortality.

Roles of Gram-positive and Gram-negative bacteria in neonatal sepsis

A Korean study compared the incidences of early-onset neonatal sepsis in cases of PPROM between two periods (1996-2004 and 2005-2012) and found that the incidences of early-onset neonatal sepsis due to Gram-positive bacteria were similar (1.5% vs 1.6%, P=1.0) between the two periods, while the incidences of early-onset neonatal sepsis due to Gram-negative bacteria were significantly different (0.6% vs 2.7%, P=0.04).[10] In our cohort, the incidence of early-onset neonatal sepsis due to Gram-positive bacteria was 3.1% (17/553), while the incidence of early-onset neonatal sepsis due to Gram-negative bacteria was 2.9% (16/553). An Israeli study investigated patients with preterm delivery (<37 weeks of gestation) who had maternal fever, chorioamnionitis, or PPROM; the results showed that early-onset neonatal sepsis was caused by *E coli* in 80% of patients (12/15), whereas it was not caused by GBS in any patients.[9] In our cohort, the incidences of early-onset neonatal sepsis caused by GBS and *E coli* were both 2.0% (11/553). However, the presence of *E coli* in maternal, placental, or neonatal specimens was significantly associated with the development of early-onset neonatal sepsis (P=0.004); this relationship was not observed with respect to GBS (P=0.39). This was likely because of the prophylactic erythromycin that was administered when patients were diagnosed with PPROM and the benzyl penicillin administered when these patients were in labour; these antibiotics were able to partially control GBS, but were generally unable to control *E coli*. Therefore, it is important to administer an antibiotic that can control both Gram-positive and Gram-negative bacteria in PPROM patients.

Insufficient control of *Escherichia coli* by ampicillin and erythromycin

There have been very few studies regarding colonisation of female genital tracts by *E coli*, especially among pregnant patients. In an analysis of 514 patients with female genital tract infections during 2016 and 2017, 17.7% of the infections were found to be caused by *E coli*; its rate of resistance to ampicillin was 67% (61/91).[12] A large study regarding the resistance of *E coli* in urinary tract infections (n=42 033) from 1999 to 2009 found that its rate of resistance to ampicillin was 58.3%.[13] Such

(33.3%), but low rates of resistance to co-amoxiclav (3.6%) and intravenous cefuroxime (14.0%). Notably, our laboratory did not routinely perform sensitivity testing of *E coli* to erythromycin because of its presumed resistance to the antibiotic.

Discussion

The incidence of PPROM was 2.63% in our cohort, which was consistent with prior reports in the

TABLE 3. Comparison of PPROM patients with and without early-onset neonatal sepsis*

Clinical characteristics and microbiological investigation	With early-onset neonatal sepsis† (n = 60)	No early-onset neonatal sepsis (n = 493)	P value (95% CI)
Gestation at PPROM (weeks)	31.1 ± 4.1	34.2 ± 2.5	<0.001 (-3.83 to -2.37)
Gestation at delivery (weeks)	31.6 ± 3.9	34.4 ± 2.3	<0.001 (-3.46 to -2.09)
PPROM to delivery interval (days)	3.7 ± 7.6	1.5 ± 3.9	<0.001 (1.05-3.44)
Gram-positive bacteria in maternal, placental, or neonatal cultures‡	17 (28.3%)	85 (17.2%)	0.036
GBS in maternal, placental, or neonatal cultures‡	11 (18.3%)	70 (14.2%)	0.39
Gram-negative bacteria in maternal, placental, or neonatal cultures‡	16 (26.7%)	55 (11.2%)	0.001
E coli in maternal, placental, or neonatal cultures‡	11 (18.3%)	36 (7.3%)	0.004
Anaerobes in maternal, placental, or neonatal cultures‡	3 (5.0%)	7 (1.4%)	0.08

Abbreviations: CI = confidence interval; E coli = Escherichia coli; GBS = group B Streptococcus; PPROM = preterm prelabour rupture of membranes
* Data are shown as mean ± standard deviation or No. (%) of patients, unless otherwise specified
† For twin pregnancies, if either one of the neonates or both had early-onset neonatal sepsis, that pregnancy was considered to have early-onset neonatal sepsis
‡ Four patients with mixed bacterial growth had early-onset neonatal sepsis and all cultured bacteria were included for analysis

data were consistent with our findings that 70.3% of E coli isolates were resistant to ampicillin. With the exception of a meta-analysis in Ethiopia that showed 52.9% of E coli isolates were resistant to erythromycin,[14] very few studies in the literature have investigated the extent of E coli resistance to erythromycin. It is generally believed that E coli isolates are intrinsically resistant to low-level macrolide antibiotics due to plasmid-mediated resistance; this includes a high rate of resistance to erythromycin.[15] Therefore, erythromycin is rarely used to treat E coli infection, and most laboratories, including our centre, do not routinely perform erythromycin sensitivity testing for E coli.

However, most international guidelines[5-8] currently recommend the use of erythromycin, with or without ampicillin, in PPROM patients. Based on the findings in our study, this antibiotic regimen does not provide adequate control of E coli, as it was most frequently identified as the cause of early-onset neonatal sepsis.

Insufficient control of Group B Streptococcus by erythromycin

Group B Streptococcus was detected in 14.6% of maternal, placental, and neonatal specimens in our cohort; similarly, in a study published in 2014, Yeung et al[2] reported a GBS maternal carrier rate of 12.5% in PPROM patients. They found that the rate of resistance to erythromycin was 65% among GBS isolates, and that the incidence of neonatal GBS infection was significantly lower in patients who received penicillin than in those who received erythromycin (0.0% vs 36.4%; P=0.012). In addition, Yeung et al[2] suggested that, instead of erythromycin, ampicillin or amoxicillin should be administered to PPROM patients who are active GBS carriers or whose GBS status is unknown. Although our cohort demonstrated a lower rate

TABLE 4. Proportions of cultured Gram-positive bacteria that were resistant to erythromycin or penicillin

Gram-positive bacteria	Erythromycin resistance	Penicillin resistance
Group B Streptococcus	19/45 (42.2%)	0/77
Other Streptococcus	4/8 (50.0%)	0/8
Enterococcus	2/4 (50.0%)	Not available
Staphylococcus	0/2	Not available

TABLE 5. Proportions of cultured Gram-negative bacteria that were resistant to various antibiotics

Gram-negative bacteria	Ampicillin resistance	Co-amoxiclav resistance	Gentamicin resistance	Cefuroxime (intravenous) resistance	Cefuroxime (oral)	
					Resistance	Intermediate sensitivity
Escherichia coli	26/37 (70.3%)	1/28 (3.6%)	14/42 (33.3%)	6/43 (14.0%)	5/44 (11.4%)	14/44 (31.8%)
Klebsiella	8/9 (88.9%)	0/7	0/8	0/7	0/9	2/9 (22.2%)
Proteus	4/4 (100.0%)	1/3 (33.3%)	1/4 (25.0%)	1/3 (33.3%)	NA	NA

Abbreviation: NA = not available

of resistance (42.2%) of GBS to erythromycin, we agree that the administration of erythromycin alone is insufficient to control GBS. In addition, our findings demonstrated that approximately half of the other Gram-positive bacterial isolates, including *Enterococcus* and other *Streptococcus* bacteria, were resistant to erythromycin; thus, the overall number of Gram-positive bacterial isolates controlled by erythromycin could be as low as 42.4% (25/59).

Potential use of other antibiotics and clinical implications

Escherichia coli was found to have a low rate of resistance to co-amoxiclav (3.6%); GBS was also expected to be sensitive to co-amoxiclav because none of the GBS isolates were resistant to penicillin in our cohort. However, the ORACLE 1 trial found that the use of co-amoxiclav in PPROM patients was significantly associated with an increased incidence of neonatal necrotising enterocolitis, compared with the use of other antibiotics (1.8% vs 0.7%, P=0.0005).[1] Most international guidelines discourage the use of co-amoxiclav because of this finding.[5-8] A Cochrane systematic review in 2013 assessed 22 randomised controlled trials regarding the use of prophylactic antibiotics in PPROM; only three small trials had compared the incidence of neonatal necrotising enterocolitis between placebo and other penicillins that were not co-amoxiclav.[3] Two trials investigated the use of mezlocillin (n=47 and n=40), whereas the other investigated the use of piperacillin (n=37); none found an increased incidence of neonatal necrotising enterocolitis.[16-18] Mezlocillin is no longer available in the market as it has been replaced by other penicillins with better bacterial coverage such as piperacillin and ticarcillin. Piperacillin is typically reserved for more severe infections that are resistant to cephalosporin; thus, it is seldom prescribed as first-line treatment. Therefore, piperacillin may not be suitable for use as antibiotic prophylaxis in asymptomatic PPROM patients without evidence of acute chorioamnionitis. In our cohort, *E coli* had a 14.0% of rate of resistance to intravenous cefuroxime, and *Klebsiella* showed no resistance; thus, intravenous cefuroxime could be appropriate for controlling both Gram-positive and Gram-negative bacteria. Thus far, there have been no studies regarding the use of cefuroxime in patients with PPROM. A large study regarding antibiotic resistance rates of *E coli* isolates in urinary tract infections (n=42 033), from 1999 to 2009, found that the rate of resistance to cefuroxime was 3.7%.[13] Finally, one third (33.3%) of *E coli* isolates were resistant to gentamicin in our cohort. A threshold of 20% has been suggested as the degree of resistance at which an antibiotic should no longer be used empirically.[19] Because of the resistance of *E coli* to gentamicin and its potential side-effects in terms of ototoxicity and nephrotoxicity, gentamicin is not recommended as a routine prophylactic antibiotic in PPROM.

A study in Korea published in 2016 proposed the use of a combination of ceftriaxone, clarithromycin, and metronidazole in PPROM patients, and this new regimen was shown to more frequently eradicate intra-amniotic inflammation or infection, as well as to more frequently prevent secondary intra-amniotic inflammation or infection, compared with an antibiotic regimen which included ampicillin and/or cephalosporin.[20] In our cohort, only 1.8% of maternal, placental, or neonatal specimens demonstrated growth of anaerobes, and these were not associated with early-onset neonatal sepsis. Therefore, the use of metronidazole may not be essential in PPROM patients. In the current recommendations from a variety of international guidelines, erythromycin remains the most commonly used macrolide with an established safety profile in perinatal use, relative to other next-generation macrolides, such as clarithromycin. Therefore, we recommend continued usage of erythromycin in PPROM patients, rather than clarithromycin. However, Gram-positive and Gram-negative bacteria were found in a total of 29.1% (161/553) patients with PPROM in our cohort. Based on the presumption that all Gram-negative bacterial isolates were resistant to erythromycin, the use of erythromycin alone as a broad-spectrum antibiotic regimen was insufficient for control of Gram-positive and Gram-negative bacteria in 67.7% (109/161) of these culture-positive patients, or 19.7% (109/553) of all patients with PPROM in our cohort. Therefore, additional antibiotics are needed to achieve better control of GBS and Gram-negative bacteria, particularly *E coli*. Co-amoxiclav is not recommended because it is associated with an increased risk of neonatal necrotising enterocolitis. Based on our findings, we propose the addition of intravenous cefuroxime. Ceftriaxone, a third-generation cephalosporin, is presumed to be equally effective, or to be more effective than cefuroxime, in controlling Gram-negative bacteria. However, because of the risk of generating drug resistance in other bacteria, such as *Enterobacter*,[21,22] third-generation cephalosporins may not be suitable for use in empirical antibiotic prophylaxis in asymptomatic PPROM patients without evidence of acute chorioamnionitis.

Oral cefuroxime is more convenient to prescribe than intravenous cefuroxime in PPROM patients. However, Gram-negative bacteria were more sensitive to intravenous cefuroxime than oral cefuroxime in our cohort: 31.8% of *E coli* and 22.2% of *Klebsiella* only showed intermediate sensitivity to oral cefuroxime. Therefore, we recommend the administration of a 1-week course of intravenous cefuroxime in PPROM patients, combined with 10 days of oral erythromycin. Furthermore, the efficacy

of a combined regimen, such as 3 days of intravenous cefuroxime followed by 4 days of oral cefuroxime, together with oral erythromycin, needs additional analysis to determine whether they are comparable in PPROM patients.

Limitations of this study

There were some limitations in our study. First, our hospital laboratory did not perform sensitivity testing of Gram-negative bacteria (including *E coli*) to erythromycin, because of its assumed resistance. Second, our hospital laboratory did not perform sensitivity testing of all isolated pathogens; instead, it performed testing of pathogens with significant growth in culture, and such testing was limited to the most commonly used antibiotics. Therefore, the full spectrum of sensitivity of identified pathogens to various possible antibiotics could not be fully established from the available data. Because of our departmental guidelines for prescribing intrapartum benzyl penicillin for patients with preterm labour, the clinical outcomes observed in our cohort—particularly with regard to early-onset neonatal sepsis—could have been influenced by the combined use of erythromycin and intrapartum benzyl penicillin, rather than by the effect of erythromycin alone.

Conclusion

Use of erythromycin with or without ampicillin was insufficient to control Gram-positive and Gram-negative bacterial growth in patients with PPROM. Based on the increase in Gram-negative bacteria and the association of these bacteria with the development of early-onset neonatal sepsis, intravenous cefuroxime (a second-generation cephalosporin) is proposed for use as antibiotic prophylaxis, in combination with erythromycin. Further studies regarding the use of erythromycin combined with intravenous cefuroxime in PPROM patients are suggested to investigate the efficacies of these antibiotics for preventing early-onset neonatal sepsis, and to explore their side-effects, such as the development of neonatal necrotising enterocolitis.

Author contributions

All authors had full access to the data, contributed to the study, approved the final version for publication, and take responsibility for its accuracy and integrity.

Concept or design of the study: CW Kong.
Acquisition of data: YY Li.
Analysis or interpretation of data: All authors.
Drafting of the article: YY Li, CW Kong.
Critical revision for important intellectual content: WWK To.

Conflicts of interest

All authors have disclosed no conflicts of interest.

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